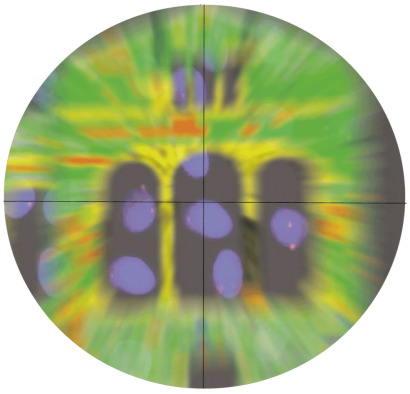
ISDQP

XIVth ISDQP INTERNATIONAL CONGRESS

DIAGNOSTIC MOLECULAR PATHOLOGY: FROM GENOMICS TO PROTEOMICS

PROCEEDINGS



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Edited by:
A. Sampedro.

FOREWORD

Interest in diagnostic molecular pathology has greatly increased in the past few years both in basic research and diagnostic point of view. This is largely due to the development of new instruments and relevant applications mainly in the areas of genomics and proteomics. The XIV ISDQP congress, held in September 2001 in Oviedo, brought together different experts in both methodological approaches. At the same time it gave us the opportunity to bring together the scientific information of the groups with a lot of experience and a well-known scientific level in these areas, to whom we are very grateful. Because of this it is a pleasure for us to present this book to those members of the scientific community who are involved in the area of diagnostic molecular pathology.

Within the first group of presentations a summing-up of sampling and tissue management as well as the significance of sampling by laser microdissection is provided. In addition, new approaches in molecular cytogentics were analysed. Recurrent chromosomal aberrations reflect genomic changes that contribute to the transformation of normal cells into tumor cells. The new molecular cytogenetic techniques (FISH, CGH, SKY) revealed a high number of previously undetected or incompletely identified chromosome aberrations.

A major accomplishment of the XXI century was celebrated in the month of February 2001 with the publication of the nearly complete sequence of the human genome. Any initial attemp to catalog those relevant gene players/markers require to draw complete expression profile maps, which involves the examination of thousands of genes. The revolutionary methodology of DNA chip/microarray has developed sufficient capacity and productivity as to allow the examination of complete genomes. Integration of the functional genomic view of the cancer genome with the cytogenetic view, could lead to the identification of genes playing a critical role in cancer development and progression. Furthermore by using a combination of three different microarray technologies, cDNA, CGH, and tissue microarrays, we can directely identifiy genes involved in chromosomal rearrangements in cell line model systems and then rapidly explore their significance as potential diagnostic and therapeutic targets in human cancer progression.

The usefulness of a morphological approach for 3-D biological structures is ilustrated via three examples: about embrionic development of the rat kidney, the morphogenesis of long bones and the experimental osteoporosis. In addition, an original approach for the detections and measurements of the DNA spots in micro-arrays, allows to read large plates rapidly and with robustness.

Telepathology comprises the adquisition and transport of digitized images, the detection and extraction of information content, and its transformation into a diagnosis. The increasing use of telepathology and the existence of digitized information in pathology argues for implementation of artificial intelligence (AI) systems in application of pathology. AI systems in telepathology can be used for assessment of a dynamic quality control in diagnostic pathology, and efficient control of dynamic microscopes via the internet.

The European Commission has recently adopted (March 2001) an action plan to develop the e-learning initiative, which has as its main objective to promote lifelong learning in any field of knowledge using the World Wide Web and electronic devices.

This action plan focuses in the study of factors involved in the quality of learning through the web, as methodologies, contents, resources, pedagogical approaches, educational management or technological developments. In order to promote lifelong learning in Pathology, three main factors must be taken into account: technological resources and infraestructure, pedagogical approach for multimedia educative materials, and training of designers, training of trainers and training of students.

We would like to thank all the authors for their valuable contributions, Verónica Fernández and Ana Salas for assistance with translation and manuscript revision, and the group of Image Processing Service of the University of Oviedo for formatting the manuscripts .

Andrés Sampedro

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PLENARY SESSIONS

PS1 - TUMOR-SPECIFIC CHROMOSOMAL ABERRATIONS SERVE AS DIAGNOSTIC MARKERS IN CLINICAL ONCOLOGY AND AS RESEARCH OBJECTS IN FUNCTIONAL AND COMPARATIVE CYTOGENETICS

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Recurrent chromosomal aberrations reflect genomic changes that contribute to the transformation of normal cells into tumor cells. CGH- and SKY-analysis of human and mouse tumors revealed a high number of previously undetected or incompletely identified chromosome aberrations.

CGH serves as a screening test for DNA-copy number changes in tumor genomes. Gains or losses of specific chromosomal regions reflect increased copy numbers of oncogenes and decreased copy numbers of tumor suppressor genes. CGH is based on a two color FISH-experiment, where a normal reference genome is labeled with a first fluorochrome, e.g. rhodamine and the genomic tumor DNA with a second fluorochrome, e.g. fluorescein. The differentially labeled genomes are hybridized to normal reference metaphase chromosomes. Changes in the ratio of the fluorescein/rhodamine intensities reflect DNA-copy number alterations in the tumor. Genomic DNA is the only material required from the tumor for CGH-analysis, thus allowing for the use of archived, formalin-fixed and paraffin-embedded samples as well.

SKY permits the simultaneous visualization of all chromosomes in a metaphase spread in different colors. Structural chromosomal aberrations that lead to the formation of new fusion genes or to oncogene activation are readily identified. SKY is based upon combinatorial FISH using chromosome painting probes, optical microscopy, spectral imaging and spectra-based chromosome classification.

The identified recurrent chromosomal aberrations that are specific for each tumor type and for different tumor stages are useful genetic markers for cancer screening tests in clinical diagnostics. Interphase cytogenetics with region-specific DNA-probes (fluorescence in situ hybridization experiments on interphase nuclei) allows for the detection and classification of tumor cells in biopsy samples, disseminated tumor cells and formalin-fixed tissue sections.

Functional cytogenetics includes for instance the identification of genetic mechanisms that lead to the generation of chromosomal aberrations. Fragile Sites might be hot spots for DNA double-strand breaks at specific sites followed by non-homologous recombination. Using SKY, DAPI and Giemsa staining techniques we screened normal human and mouse lymphocytes that were treated with aphidicolin. We identified several common fragile sites that have not been known before, e.g. on chromosome arms 2p, 5p, 5q, 6p, 8p, 9p, 9q, 14, 18q, 19p, 20q, 22. We are currently establishing high resolution maps of chromosomal regions that are frequently involved in tumorspecific chromosome translocations and located in the vicinity of common FS. Arrays of BAC clones covering these regions will be used to investigate the physical and functional association of fragiles sites and chromosome translocation breakpoints during tumor formation.

Comparative cytogenetics allows for the functional analysis of genes as part of genetic pathways. Transgenic and knockout mice that develop specific tumors and

receive selected treatments have been analyzed in collaboration with several research groups using mouse SKY and mouse CGH.

PS2 - DIFFERENCES IN STRUCTURAL CHROMOSOMAL ABERRATIONS BETWEEN TWO TYPES OF EPITHELIAL TUMORS: ORAL SQUAMOUS CELL CARCINOMA VERSUS COLORECTAL ADENOCARCINOMA.

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Chromosomal instability is the most important type of genetic instability in solid tumors. CGH analysis has greatly facilitated its investigation, reporting many recurrent chromosomal imbalances of parts of chromosomal arms, which must be related to structural chromosomal changes. However, hardly anything is known about chromosomal translocations in these tumors. The question remains wether structural chromosomal changes themselves directly contribute to cancer development, or rather their resulting gains and losses of chromosomal material. Recently, Spectral Karyotyping (SKY) has been developed using 24 chromosome specific probes that are combinatorially labeled with 5 different fluorochromes. Thus, all chromosomes are visualized with distinct fluorescent colors and are automatically classified based on their characteristic spectra. With this technique, two epithelial tumor types, colon adenocarcinoma (CAC, 10 cell lines) and oral squamous cell carcinoma (OSCC, 9 cell lines), were studied for chromosomal rearrangements.
In the CAC, no recurrent combinations were found, except isochromosomes 3q, 8q, 15q and 20q. Chromosome bands 1p36, 4q31, 8q24, 9p21, 9q34, 12q21, Xp22 were frequently involved in rearrangements, providing additional information to from those regions found by CGH in primary colon carcinomas (8, 13, 17, 18, 20). In the OSCC, rearrangents occurred in much higher numbers. Again, no recurrent combinations were found, except isochromosomes 3q, 8q, 21q, Xq, 5p, and also whole arm translocations 1q-18p, 1q-10q, 2p-11p, 4p-12p, 6p-14q, 15q-18p. Chromosome bands 1p31, 1p32, 3p21, 3q25, 4q28, 11q13, 15q15, 17p12, 18q23, 20q13.3, 22q11.2, 22q12 were frequently involved in rearrangements. Apart from differences in frequently rearranged chromosome bands, the types of translocation differed between the two tumors. CAC showed more 'band-band' translocations (62% versus 35%), whereas OSCC had more centromeric translocations (43% versus 21%). In addition, jumping translocation were seen only in OSCC.
In conclusion, colon adenocarcinoma and oral squamous cell carcinoma differ both in chromosomes involved and in type of rearrangements, which shows that structural chromosomal changes are not random and must therefore play a direct role in carcinogenesis.

PS3 - PATTERNS OF CHROMOSOMAL INSTABILITY IN COLORECTAL ADENOMA –CARCINOMA PROGRESSION

Mario Hermsen[1], Cindy Postma[1], Marjan Weiss[2], Stephan Meuwissen[2], Richard Williams[3], Walter Giaretti[4], Ana Rapallo[4], A Di Vinci[4], Ton de Goeij[5], Jan Willem Arends[5], Jan Baak[1], Gerrit Meijer[1]

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Colorectal tumor progression is driven by an accumulation of genetic abnormalities affecting critical biological pathways. These abnormalities arise as a consequence of genomic instability, in colorectal cancer occurring mainly at the chromosomal level. Current chromosomal instability models of colorectal carcinogenesis are to a major degree based on studies analysing only a limited of parameters and therefore, rather simplistic. Recently, more genome-wide genetic analysis techniques have become available, however, until now studies of colorectal adenoma to carcinoma progression have been too small to allow complex data analysis.

We have analysed a total of 194 tumors (in 4 groups: 66 simple adenomas, 46 malignant polyps, both adenoma and carcinoma parts, and 36 carcinomas) for chromosomal losses and gains throughout the genome by CGH, supplemented by mutation analysis of APC, K-ras and p53, as well as immunohistochemical analysis of p53 expression.

A first analysis showed that the number of aberrations correlated with the grade of dysplasia in adenomas, whereas only a trend was noted with histological type and size of adenoma. More relevant information was obtained by considering only seven chromosomal abnormalities that occur in more than 40% of carcinomas: losses on 8p, 15q, 17p and 18q, and gains on 8q, 13q and 20q, i.e. the 'bad events'. The number of 'bad events' correlated to dysplasia and to p53 mutation. Interestingly, the adenoma parts of malignant polyps harboured a significantly higher number of 'bad events' than the simple adenomas, also when stratified for degree of dysplasia and p53 status. This suggests that the accumulation of these seven 'bad events are indicative for risk of progression from adenoma to carcinoma. Extrapolating a linear model from these results proved difficult since no specific 'bad event' appeared to occur earlier or later in progression than another

To do justice to the complexity and heterogeneity in colorectal cancer, hierarchical cluster analysis was performed, yielding a classification of 4 clusters of adenomas and two clusters of carcinomas. When looking to the 'bad events' within these clusters, it appeared that they segregated over the adenoma clusters: no events in cluster 1, loss of 17p in cluster 2, gains of 8q and 13q in cluster 3, and loss of 18q and gain of 20q in cluster 4. Adenoma clusters 2 and 4 showed great similarity with carcinoma clusters 1 and 2, respectively, whereas adenoma cluster 3 had 'bad events' in common with both carcinoma clusters.

This suggests that a specific combination of 'bad events', rather than an accumulation, is sufficient for malignant progression. Furthermore, it would appear that at least three separate genetic pathways from colorectal adenoma to carcinoma exist, each involving a different set of chromosomal changes.

PS4 - CDNA MICROARRAYS IN CANCER. CONCEPTS AND APPLICATIONS

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Only a fraction of the estimated 32,000 human genes is activated in any given tissue at a given time. The level of gene activation can greatly vary with different physiological stimuli and determines the behavior of the biological tissue or cell subset.

In cancer, as in any other situation, the overall gene expression level known as transcriptional profile reflects the genetic program activated in the cell or tissue and decides both resistance and sensitivity to therapy. Cancer heterogeneity that escapes current classifications is the main cause of failed responses to clinical treatment. It is hypothesized that at least for certain types of cancer knowing the transcriptional profile (of a particular tumor) a patient specific therapy could be selected to maximize efficiency.

It might be sufficient to look at a limited number of informative genes to predict response categories, but reliable markers are unknown yet to subclasify most cancer types. Any initial attemp to catalog those relevant gene players/markers require to draw complete expression profile maps, which involves the examination of thousands of genes.

Classical analytical tools lack the needed capacity to address this issue. In the last five years the revolutionary methodology of DNA chip/microarray has developed offering sufficient capacity and productivity as to allow the examination of complete genomes. DNA microarrays acquire different formats and can cover different applications, from transcriptional profiling to genotyping.

The Spanish National Cancer Center (CNIO) has implemented the cDNA microarray technology to address questions on cancer development and response to treatment in particular tumor types. The principal objective is to help predict clinical behavior by identifying tumor specific marker genes. The CNIO's human Oncochip as well as other developments for gene expression analyses will be presented and potential applications discussed.

PS5 - THE BIOINFORMATICS OF DNA ARRAYS DATA ANALYSIS

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Bioinformátics Unit, CNIO

New methodologies for genomic analysis, like DNA arrays, provide a huge amount of data of such a complexity that its analysis and management would be impossible without a solid bioinformatics support.

A proper identification of the bottlenecks and the critical steps in the analysis allows the implementation of appropriate bioinformatics solutions. Bioinformatics is a necessary tool in the different steps of the DNA microarray experiments, from the design of the microarray, including the storage of the results and quality control checkpoints, and especially in the final phase of analisys of results.

The first bottleneck constitutes the design of the microarray itself, that is, to decide which genes will be included in it. Usually, brute force approximations, that implied the inclusion of as many genes as possible in the microarray, have been used. This is neither desirable nor possible in many cases, being a better solution including a bunch of genes carrefully selected for the intended experiment. A database including information about function, tisue in which the genes are expressed, etc., allows an appropriate selection of the genes (and the corresponding clones) for the microarray.

Data storage is another key step because the volume of data produced is not negligible. It is necessary to use a relational schema for the database that allows a quick and efficient management of the data stored. Quality control systems link results to the design. If, for some reason, a clon of a gene fails, this fact must be annotated to be taken into account automatically for the next time a clon is going to be selected for this gene.

Finally, data analysis is one of the field where bioinformatics is more active. Apart from the software that converts the signal of the labeled sample into a ratio of differential gene expression, there are diverse programs and algorithms for analysing the results. Clustering algorithms, either for finding genes that coexpress or conditions with similar gene expression patterns are among the most popular tools for analysing the results of microaray experiments. Datamining methodologies provide a new way for analysing the results gathering information dispersed among sifferent databases and extranting common patterns for groups of genes that cluster together under a given experimental condition. This is one of the most promising areas in bioinformatics.

PS6 - EXPRESSION MICROARRAYS IN THE PREDICTION OF TREATMENT RESISTANCE: THE CNIO PROGRAM

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The Molecular Pathology Program in the Spanish National Cancer Research Centre (CNIO) has the objective of to perform molecular analysis of human tumours, which could allow to predict the treatment response to commonly used drugs, permitting at the same time the identification of relevant genes in cancer.

Biologic therapy has been so far based in the study of unique genes or proteins, such as the administration of Tamoxifen to ER positive tumours, Herceptin for c-erb-B2 overexpressed cancer, Cetuximab for EGF overexpressed cancers, Rituximab in CD20 positive lymphomas or RAR in PML. Although this biologically oriented treatment is making significant contributions to different tumoral types, it ignores the fact that cancer is a social and multigenic disease. Thus the more common forms of cancer are the result of complex genetic alterations which allow the tumoral cells to develop multiple capacities, such as tissue invasion and metastasis, angiogenesis promotion, unlimited replication, apoptosis escape, production of autonomous growth signals, insensitivity to cycle control signals and generation of genomic instability.

One of the goals of the Molecular Pathology Program in the CNIO is to contribute to an individualised therapeutic strategy, according to the multiple and varied characteristics of the disease. For this purpose, The CNIO is analysing resistance to commonly used drugs, through molecular study of targets and mRNA expression analysis using a cDNA microarrays build-up specifically with this purpose with approx. 7.000 genes and EST's, and denominated as ONCOCHIP. Additionally the CNIO is sponsoring the creation of a Network of Tumour Banks in the main Hospitals, which allow a massive application of these molecular techniques in large series of patients diagnosed and treated using standardised protocols.

Some of the projects currently in realisation are the following:

- *Study of the molecular mechanisms of therapeutic fail in CTCL and MM treated with α-interferon and/or PUVA.* The objective of this project is to identify the genes involved in α-IFN resistance in MF and MM.
- *Study of the molecular mechanisms of therapeutic fail in CML treated with α-interferon and/or STI571.*
- *Development of a cDNA biochip for the analysis of molecular changes associated with treatment resistance in breast cancer.*

The CNIO is open to establish collaborations with other co-operative groups in Cancer Research, to perform integrated analysis of molecular factors predicting treatment resistance.

PS7 - BIOCHIP TECHNOLOGIES IN IDENTIFYING CANDIDATE GENES IN CANCER

Outi Monni

Biomedicum Biochip Center, Finland

A vast number of recurrent chromosomal alterations have been implicated in cancer development and progression. However, most of the genes involved in recurrent chromosomal alterations in solid tumors remain unknown, despite the recent substantial progress in genomic research and availability of high-throughput technologies. For example, it is now possible to quickly identify large numbers of differentially expressed genes in cancer specimens using cDNA microarrays. Integration of this "functional genomic view" of the cancer genome with the "cytogenetic view" could lead to the identification of genes playing a critical role in cancer development and progression. By using a combination of three different microarray technologies, cDNA, CGH, and tissue microarrays, we can directly identify genes involved in chromosomal rearrangements in cell line model systems and then rapidly explore their significance as potential diagnostic and therapeutic targets in human primary breast cancer progression.

We are applying cDNA and CGH microarray technologies to the analysis of specimens where molecular cytogenetic analysis has identified consistent chromosomal alterations, such as DNA amplifications. We have constructed high-density cDNA microarrays for the comprehensive analysis of amplifications along chromosome 17 in breast cancer. We initially targeted the *HER-2* amplicon at 17q12-21 and the 17q23 amplicon identified by CGH as a recurrent DNA amplification site in breast cancer. We have used these cDNA microarrays for parallel gene copy number and gene expression analyses in breast cancer cell lines. A limited number of genes that are both amplified and overexpressed at 17q23 have been identified. Also, our results indicate that a number of genes besides the *HER-2* oncogene are highly amplified and overexpressed at the 17q12-q21 region. These limited numbers of candidate amplification target genes at each region can now be functionally analyzed to identify phenotypic effects that the activation of these genes may have on breast cells.

After identification of candidate genes in cell line model systems one can use tissue microarray (TMA) technology to translate such findings to the in vivo situation, for example to explore the clinical significance of newly discovered molecular alterations as diagnostic and therapeutic targets. TMAs enable rapid analysis of molecular targets in thousands of tissue specimens either at DNA, RNA or protein level. TMAs are constructed by acquiring 0.6 mm cylindrical core specimens from up to 1000 histologically defined tissue specimens and arraying them at high density into a TMA block. Up to 300 consecutive sections can then be cut from each TMA block allowing the analysis of a large set of markers in the same series of samples. For example, one of the genes discovered as amplified and overexpressed in breast cancer cell lines with the cDNA and CGH microarray surveys was the ribosomal protein S6 kinase gene (*RPS6KB1* at 17q23), which codes for a critical mediator involved in G1 to S-phase progression. In breast cancer cell lines, *RPS6KB1* amplification led to increased mRNA and protein expression as well as increased kinase activity. We therefore decided to test the involvement of the *RPS6KB1* gene in vivo in human primary breast cancer. Using the tissue microarray technology we showed by fluorescence in situ hybridization that *RPS6KB1* was amplified in 8.8% of the 668

primary breast tumors. Furthermore, the *RPS6KB1* amplification was associated with poor prognosis of the patients. *RPS6KB1* amplification occurred independently of the *HER-2* amplification, a known prognostic indicator in breast cancer. Finally, co-amplification of the *RPS6KB1* and *HER-2* genes implied a worse survival than amplification of either one alone.

In conclusion, combination of cDNA, CGH and tissue microarray technologies allow rapid identification of candidate genes in cancer. With the availability of the human genome sequence, cDNA microarrays with full representation of all transcripts from a specific chromosomal region can be constructed and applied in CGH and cDNA microarray analyses of cytogenetically characterized cancer tissues and cell lines. This leads to the integration of the cytogenetic and functional genomic views of the cancer genome, which facilitates the deeper mechanistic understanding of the molecular basis of cancer development, more than could be achieved by either research technique alone. Finally, tissue microarrays are likely to be critical for the clinical studies of all of the thousands of novel genes and proteins identified in large-scale screening studies of the human genome and proteome.

PS8 - HIF-1a IN BREAST CARCINOGENESIS, TUMOR PROGRESSION AND CLINICAL BEHAVIOUR

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Hypoxia Inducible Factor 1 (HIF-1) is a transcription factor thought to play an important role in tumor growth and metastasis by regulating cell metabolism and inducing angiogenesis in order to survive cellular hypoxia. Overexpression of the HIF-1α subunit in different human cancers has already been demonstrated. Using immuno-histochemistry on paraffin embedded sections, the expression of HIF-1α at different stages of breast carcinogenesis was determined and correlated with VEGF, HER-2-neu, p53, Ki-67, estrogen receptor (ER) status and microvessel patterns, in the six major diagnostic categories of breast lesions. Neither normal breast tissue (N=10) nor ductal hyperplastic lesions (N=10) showed HIF-1α overexpression. Overexpression was found in 4/10 well differentiated ductal carcinomas in situ (DCIS) and 6/10 well differentiated invasive breast cancers. Also the poorly differentiated cases showed an increase in HIF-1α overexpression from DCIS (8/10) to invasive cancer (10/10). HIF-1α overexpression was positively correlated with Ki-67, and VEGF. HER-2-neu, p53 and microvessel density were not significantly correlated although a positive trend with HIF-1α overexpression was seen.
In a larger follow up study, comprising 153 breast cancer patients, high levels of HIF-1α were independently strongly associated with decreased disease-free, and overall survival. This effect could completely be ascribed to the subgroup of lymph node negative cancers (N=81). There was now a strong correlation between HIF-1 expression and HER-2/neu overexpression.
In conclusion, HIF-1α overexpression occurs already in DCIS, indicating that HIF-1α may play a role in breast carcinogenesis. Moreover, HIF-1α overexpression is associated with increased proliferation and poor differentiation grade in breast lesions, and is indeed associated with a poor prognosis in lymph node negative breast cancer patients. Thus, immunohistochemical assessment of HIF-1α, may improve clinical decision-making regarding adjuvant treatment of lymph node negative breast cancer patients and justifies the development of anti-HIF-1α therapeutics in breast cancer.

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PS9 – QUANTITATIVE ANALYSIS OF IMMUNOHISTOCHEMISTRY FOR PROGNOSTIC PURPOSES

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Quantitative evaluation of any staining is dependent on the relationship between the amount of stained molecules and the amount of stain deposited after the staining. Stoichiometric staining will result in a linear standard curve. Immunohistochemical staining aims to this but there are numerous reasons, why true stoichiometric relationship is not reached. However, larger number of stainable molecules in the sample will results in more intense staining also in immunohistochemistry. The latter fact allows us to seriously consider the quantitation of immunohistochemical staining. Subjective analysis into several classes is the classical approach. However, there is interobserver variability, which can be improved by automatic analysis of staining. Generally automatic solutions should be developed for each antigen separately. The use of a staining index, which will incorporate the staining intensity and the extent of staining (Lipponen & Collan: Acta Stereol 11:125-132, 1992) has proven to be helpful in several situations. The approach has made it possible to find diagnostically meaningful antigens such as cathepsin H in distinguishing renal oncocytomas and carcinomas (Castren et al. Anticancer Res 20:537-540, 2000). Also other types of prognostic associations have been proven. The latter include cystatin A - a marker for aggressive breast cancer (Kuopio et al. Cancer Res 58:432-436, 1998). E-cadherin immunostaining and her2 (erbB2) immunostaining in breast cancer - when analysed with the index - divided the studied samples into prognostic groups at a higher level of significance than the traditional evaluation did. The index approach, which can also be automated for each antigen separately, is often surprisingly reproducible. The staining characteristics of some antigens, such as bcl-2 in breast cancer , do not allow improvement of evaluation by the index approach (Jalava et al. Anticancer Res 20:1213-1220, 2000), however.

PS10 - 3-D MORPHOLOGY IN BIOLOGY AND MEDICINE

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In this lecture, we would like to show, via three examples, the usefulness of a morphological approach for 3-D biological structures. On purpose, the examples use three different modes of acquisition, namely confocal microscopy, slicing and micro-tomography.

The first one starts from a study of Dr. Bertram, (nephrologist, dpt. of anatomy, Un. of Melbourne) about embryonic development of the rat kidney. He takes advantage of the property of embryonic kidney to develop in vitro, which enables him to study the organ evolution by confocal microscopy without animal destruction. The structure develops in the form of a tree. The expected morphological description bears on the geometry of this tree, and involves two objects :

- *extremities* : where are they located ? how are they arranged in space ?
- *branches*:where are they located ?according to which hierarchy and length ?

The second study stems from works of Dr. Staub (Laboratoire de recherches orthopédiques - hopital St Louis, Paris) about the morphogenesis of long bones, and work on the shinbones of chicken embryos. He designed a dynamic model of the long central zone (shaft), where the compact future bone appears as a series of nested co-axial cylinders
The experiment consisted in slicing the shinbone shaft, perpendicularly to its axis, into a series of a hundred semi-thin sections. Is it possible to test the model, i.e. to segment the concentric cylinders of the bone, and to describe them in quantitative terms (thickness, porosity, contacts between cylinders, etc ...)?

The third study was asked us by the European Synchrotron Radiation Facility, Grenoble, a company which produces micro-tomographs, and which had to analyse osteoporosis provoked on the mouse. The two bones investigated are the humerus and the calcaneum. The first problem here is to threshold correctly, end the second to design convenient descriptors of the thickness for the 3-D bone structure.

The lecture will end by a brief presentation of another subject: the detections and measurements of the DNA spots in micro-arrays. An original approach, mainly due to J. Angulo allows to read large plates rapidly and with robustness.

SPECIAL PRESENTATIONS

SP1 – THE ROLE OF THE PATHOLOGIST IN THE MOLECULAR ERA

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Molecular techniques play an important role in medicine today, especially in oncology, but also in a growing number of non-oncological diseases. Molecular techniques are applied within different laboratory specialities such as pathology, haematology, microbiology, and clinical chemistry.

Since some molecular techniques are non-morphological, one could argue what the role of the pathologist really is when these techniques are applied. Pathology is however a rapidly changing speciality, where new techniques have rapidly been introduced over the last few decades. These include, besides molecular techniques, also new imaging techniques, quantitative techniques, and immunohistochemistry. In the near future, microarray techniques will further revolutionarize pathological diagnosis.

Besides, many molecular techniques will be performed on tissue or cells that are primarily sent to the pathology lab, so molecular techniques naturally reside within pathology as well. Usually only after a morphological diagnosis, there will be an indication for additional techniques. For molecular techniques, this includes e.g. detection of microorganisms or screens for (cyto)genetic changes. Morphological techniques such as FISH or RNA/DNA *in situ* hybridization, or *in situ* PCR, require morphological knowledge for adequate interpretation, which is the field of the pathologist. Moreover, for non-morphological techniques, it is essential to know what kind of tissue or cells are being submitted to molecular analysis, to prevent bad results (the garbage in—garbage out principle). Adequate morphological control by e.g. sandwich H&E sections of all material submitted to non-morphological molecular techniques is therefore essential.

Of course, these principles apply just as well for the many research areas where molecular techniques are applied. Altogether, it is therefore clear that pathologist will play an increasing role in the era of molecular techniques. Molecular techniques will revolutionarize pathology and make this specialty thereby even more exiting than it is today.

SP2 – TUMOR HETEROGENEITY IN MENINGIOMAS BY FISH : IMPACT ON DISEASE OUTCOME

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Spain

In the past decades, important information has progressively accumulated as regards the cytogenetic abnormalities present in meningioma tumors and several different non-random alterations have been identified. In spite of this, at present information on the genetic heterogeneity of meningiomas is still relatively scanty. In order to assess both the inter-tumoral and intra-tumoral heterogeneity we have analyzed by interphase FISH the incidence of numerical abnormalities of 10 different human chromosomes and the association between them in a group on 70 consecutive meningioma tumors; this analysis included chromosomes 1, 9, 10, 11, 14, 15, 17, 22, X and Y. An additional goal of our study was to explore the potential clinical and prognostic implications of the abnormalities detected. From the methodologic point of view interphase FISH analysis of single cell suspensions obtained from fresh tumor samples at diagnostic surgery were used. All 70 tumors were classified from the histopathologic point of view according to the WHO classification. At the time of closing the study median follow-up was of 108 months.

Our results show that the overall incidence of numerical abnormalities for the chromosomes analyzed was 76%. Chromosome Y in males and chromosome 22 in the whole series were the most common abnormalities (46% and 61%, respectively). Despite the finding that monosomy of chromosome 22/22q$^-$ deletions are the most frequent individual abnormality (53%), in this study we have observed that chromosome gains are significantly more common than chromosomes losses (60% versus 40%). Chromosome gains corresponded to abnormalities of chromosomes 1 (27%), 9 (25%), 10 (23%), 11 (22%), 14 (33%), 15 (22%), 17 (23%), X in females (35%) and males (23%) while chromosome losses apart from chromosome 22 frequently involved chromosomes 14 (19%), X in males (23%) and Y in males (32%). Although an association was found among most gained chromosomes on one side and chromosome losses on the other side, different association patterns were observed. Furthermore, in the latter group, monosomy 22/22q$^-$ was associated with monosomy X in females while monosomy 14/14q$^-$ was associated with nulisomy Y in males. In addition, chromosome losses usually involved a large proportion of the tumor cells while chromosome gains were restricted to small tumor cell clones, including tetraploid cells. From the clinical point of view numerical abnormalities for most of the chromosomes analyzed were significantly ($p<0.05$) associated with the histopathologic subtype (chromosome 1, 9, 10, 11, 14, 15, 17, 22 and X), the DNA ploidy status (chromosomes 1, 9, 10, 11, 14, 15, 17, 22 and X) the proliferative rate of tumor cells (chromosomes 1, 9, 10, 15, 17 and X) and a shorter disease-free survival (chromosome 14 and 22). Multivariate analysis of prognostic factors showed that gains of chromosome 22 and 14q$^-$ abnormalities were the best combination of variables for predicting the patients´outcome.

In summary our results show that meningiomas are genetically heterogeneous tumors which display different patterns of numerical chromosome changes as assessed by interphase FISH. Interestingly these abnormalities usually correlate with adverse

prognostic features of the disease even when they are only present in relatively low represented tumor subclones.

SP3 – THE CHALLENGE OF PREDICTIVE MEDICINE: POTENTIAL OF CYTOMICS FOR COMPLICATION PREDICTION IN ALLOGENEIC AND AUTOLOGOUS STEM CELL TRANSPLANTATION (SCT)+

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INTRODUCTION

Disease course prediction (>95% correct) for individual patients are usually considered impossible for the majority of diseases. The substantial clinical interest in predicting the future disease development in individual patients prompts conceptually for a search of relevant information at the cellular level because of disease generation by biochemical changes in cellular systems or organs. Considering genomics or proteomics, the high multiparametric complexity and the usual preanalytic content mixing of different cell populations in cell or tissue extracts constitute substantial drawbacks for predictive conclusions. Cytomics, in contrast, as multiparameter cytometric analysis of cellular heterogenity, access a maximum of molecular cell phenotype information resulting from cell genotype and exposure. Molecular cell phenotypes contain information on the future development (prediction) as well as on the present status (diagnosis) of patient's disease.

GOAL

Complication prediction in SCT patients by flow cytometrically determined peripheral blood leukocyte three color immunophenotypes.

METHODS

CD3/16+56/45, TCRab/CD8/45, TCRgd/CD4/45, CD57/HLA-DR/TCRab, CD8/95/TCRab, CD45RO/27L/4, CD45RO/62L/4, CD8/69/TCRab following mAb CD2&2R stimulation for CD69 induction as well as intracellular (ICx) IFN-g, IL-2, IL-4, TNF-a, IL-13, CD69 determined as TCRab/ICx/CD8 following PMA+ionomycin stimulation were analyzed in 80 SCT patients by flow cytometry at 2,3,6,9,12,18,24 months post SCT including 117 healthy donors as controls. Cell frequency as well as mean intensity, intensity ratios and average packing density of antigens were calculated by quadrant analysis from FSC/SSC autogated lympho-, mono- and granulocyte FITC/PE, FITC/CY5 and PE/CY5 histograms. The resulting 3.330 data columns per patient were classified (CLASSIF1, http://www.biochem.mpg.de/valet/classif1.html) for predictive information. Data at 2 months post SCT served as baseline for the prediction of the subsequent disease course.

RESULTS

Complication free recovery, recurrent CMV infection, chronic GvH and survival were correctly predicted in >95% of the cases. It was furthermore possible to simultaneously predict either uncomplicated recovery, CMV, GvH or CMV+GvH. The simultaneous prediction for uncomplicated/CMV/GvH/CMV +GvH depends on complication indicator parameters which are either increased or decreased as opposed to complication discriminator parameters which are differently affected for different complications.

CONCLUSION

Data pattern analysis provides standardized classifiers for the early identification of SCT risk patients. The predictions provide information for earlier therapeutic intervention in risk patients as a new potential for improvement of overall clinical recovery. The predictive data patterns seem important for understanding the cellular pathogenesis of clinical complications

SP4 - SUPPORT SERVICES FOR CANCER DIAGNOSIS

A Orfao

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In January 2000 the new building of the Cancer Research Center (CIC) at the campus of the University of Salamanca was opened and Cancer research activities taking place in Salamanca have been centralized in it up now 12 differents groups with more then one hundred researchs have been incorporated into the Cancer Research Center. Based on it hundred papers have been published so far. Appart from its research activities in both the basic, translational and clinical areas, within the CIC several different programs have been created and developed which are of interest and direct application in the clinical area. Among others these include a tumor bank, a Molecular and Cellular Diagnostic Laboratory, a Unit for the genetics screening and diagnosis tumors with an hereditary component and Central Services where new technologies such as those involving genomics and proteomics are centrally available for the scientific community.

Since September 1998, the CIC has been setting up and runing a tumor bank for the storage of tumor cells and specimens with a view to prognostic research for diagnostic and therapeutic ends in cancer. This bank receives, processes and stores, for later use, biological samples of both solid tumors and haematological neoplasms corresponding to different moments in the evolution of the disease (diagnosis, remission, relapse) and different tumor specimens (primitive tumors and/or metastasis). Other activities of this unit include training courses for technical staff. The activities of the Molecular and Cellular Diagnostic Laboratory include from conventional morphologic/histologic to immunophenotypic, molecular and cytogenetic diagnosis, more then 10 samples day being currently processed in it. Genetic screening of hereditary breast cancer has been set up in collaboration between the CIC and the Instituto Mixto de Biologia Molecular from the University of Valladolid / CSIC, as a program for the in habitants of Castilla-Leon. Finally the genomic/proteomic program has started its activities recently with the goal of offering new genetic molecular diagnosis of unprecented resolution based on the biochip/microarray technology. At present the first results have been already obtained from generation to hibridization and reading of biochips.

In this presentation and after a general introduction to the current and future activities of the CIC, specific reference to the activities of the above listed clinically oriented units will be made.

SP5 - SAMPLING AND TISSUE MANAGEMENT

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The transfer of information from basic cancer research to the clinical sphere requires human neoplastic tissue. Studies carried out with cell lines and animal models are developed according to particularly standardised procedures. However, the processes of tissue handling involved in studies of human neoplastic tissue are usually more variable especially when they involve several hospitals, as the use of tissues for subsequent research is always constrained by its primary aim, which is anatomopathological diagnosis.

The need to use human neoplastic tissue under ideal conditions is currently of particular importance due to the development of:

a. Techniques of molecular pathology that allow very large-scale studies of genetic expression to be made that are also of clinical significance
b. Studies and clinical trial with the participation of numerous hospitals centres.

The Tumour Bank Network (TBN), instigated and coordinated by the Molecular Pathology Program (PPM) of the CNIO aims to respond to this need by the promotion of Tumour Banks in Spanish hospitals. This will achieved through the application of homogeneous procedures for the collection, processing, storage and shipping of neoplastic and normal tissue samples in such a way as to make it possible to carry out molecular studies that avoid the intrinsic bias of multi-centre studies.

These Hospital Tumour Banks are installed in the Pathology Department of the collaborating Hospitals, these being interconnected through a computer-based network. In this way, each Centre's tissue remains in the Hospital itself, thereby playing a key role in the development of the welfare, teaching and research activities within the Hospital. However, at the same time, it represents a tool for the encouragement of multi-hospital cancer research and of cooperation between basic and clinical researchers.

Thus, the current design is not of a Central Tumour Bank, but rather a cooperative and coordinated Network of Hospital Banks, based on simple, homogeneous and optimal tissue treatment protocols. This Network is promoted by the CNIO, which thereby undertakes the work of co-ordinating the network, using and maintaining the database, and carrying out quality control.

SP6 - THE TUMOR BANK AS A BRIDGE

Aurora Astudillo and Teresa Hernandez

Tumor Bank of HCA-IUOPA (Oviedo)

Asturias Principality has recently created the Oncology University Institute which has been officially approved on year 2000. The Institute recruits almost all the Oviedo University professionals involved in oncology research. In total, it has 70 staff members and 27 people on contractual basis. The members have different academic categories, most of them being doctors in Biology, Biochemistry, Chemistry, and Medicine, and also technicians of different categories.

At the moment, the Institute does not have a central place, but its members are working physically very closed to each other since the Hospital, and the Bioscientific Departments are placed at the same geographic area of the city. Weekly scientific meetings and periodical board sessions guarantee communication between IUOPA members, grouped in 10 different oncological research lines. Members are equally distributed in clinical (50) and basic (47) research, covering areas like: Genotoxicity and Mutagenesis, Hormonal Regulation, Molecular Biology of Cancer, Cellular Regulation, Epidemiology, Receptors and Mediators, Cellular Markers and New Technologies, Biosynthesis of new Antitumoral Agents, Clinical Research and Surgical Research.

One of the Institute goals is to approach basic and clinical research. In this sense, a Tumor Bank has been created this year (2001) located at the University Hospital of Oviedo (HCA).

Asturias has one million inhabitants, and counts with a historic Population Registry of Tumors, with an annual incidence of 4500 new cases per year; 75% of them are diagnosed and/or treated at the HCA. Given the high number of these cases going through surgery, it can be expected that about 900 tumors per year with fresh tissue, excedent of pathologic diagnosis, will be able to be included in the Bank under optimal conditions. Furthermore, it is foreseen that, whenever possible, the bank samples will incorporate a normal tissue counterpart from the same organ.

The Tumor Bank (HCA-IUOPA) started on the first of March 2001, under the initiative of the IUOPA. In these first six months, we have gathered 150 tumor samples and 100 paired normal tissues, which are stored under optimal conditions and have a corresponding sample included in paraffin. The bank has a program of courses and interviews to inform the members of the health community about the objective of this type of Banks.

For 2002, we intend to improve the number of cases, to implement a systematic quality control, and mainly to contribute setting-up a bridge between basic and clinical research with new Research Projects in common with the Institute members. We also hope to join some of the National Networks of Tumor Banks.

SP7 - LASER MICRODISSECTION IN DIAGNOSTIC MOLECULAR PATHOLOGY

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In modern medicine and biology homogeneous cell clusters or single cells for detailed analyses are required. It is most important that specimens must be prepared and captured without contamination. Even a few unwanted cells within a selected population will disturb the result of downstream molecular applications. Non-contact laser microdissection is gaining more and more importance in cell biological studies as well as in basic medical research and in diagnostic molecular pathology.
An area of application is the examination of microsatellite instability (MSI). In our institute we precisely harvested dysplastic areas (D1-D3) in cases of HNPCC by employing the first choice primer panel recommended by the NIH. These results were compared to routine molecular diagnostic procedures.
Another project is the MSI-analysis of different areas of normal crypts in HNPCC patients. Even in normal crypts MSI can be detected.
Molecular diagnostic tests are widely used in clinical medicine. In tissue specimens however false positive and false negative results can be obtained if pathomorphological and processing aspects are not considered. We therefore systematically investigated the significance of sampling by laser microdissection on three widely used diagnostic tests.

SP8 – E-LEARNING IN PATHOLOGY

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The European Comission has recently adopted (March 2001) an action plan to develop the *eLearning initiative*, which has as its main objective to promote lifelong learning in any field of knowledge using the Worl Wide Web and electronic devices. This action plan focuses in the study of factors involved in the quality of learning through the web, as methodologies, contents, resources, pedagogical approaches, educational management or technological developments.

It is the objective of our presentation to introduce some ideas about issues related with these factors and to promote discussion in the audience in order to clarify ideas and expectations about the advantages of eLearning for lifelong learning in Pathology.

Our approach in this field is that in order to promote eLearning and its Institutional Integration at Universities or at other educative contexts, three main factors must be taken into account: 1) technological resources and infrastructure, 2) pedagogical approach and multimedia educative materials, and 3) training of designers, trainers and students.

TECHNOLOGICAL RESOURCES AND INFRASTRUCTURE

Technical and functional characteristics of both, the server-client platform and the management system shall be taken into account to develop teaching-learning processes through the web (Britain and Liber, 2000; Sampedro et al., 2001). Among the issues to be considered in this area are:

Facility of access and use Versatility Motivation potencial Navigation facilities Communication tools
Technical support for structuring courses, resources and learning tasks, processes and contents
Integration with other management systems

Moreover these previous and necessary technological devices, educative institutions and individuals would need to be technologically equiped and ready to used them; that is to say, they need to have appropriate computers and operative systems, access to internet, electronic e-mail address and other resources. Many times, e-Learning experiences cannot be developed or implemented properly in educative contexts due to limitations on these matters.

PEDAGOGICAL APPROACH AND MULTIMEDIA EDUCATIVE MATERIALS (MEMs)

One of the most important factors to be taken into account when talking about learning is the pedagogical approach through which teaching and learning processes operate. The pedagogical approach condition not only the teaching methodology but also the conception of the student's and teacher's role in the teaching-learning

process, as well as the products and quality of learning. So far, it has been proved and accepted by experts in eLearning that the *student-centered pedagogical approach*, which promotes active participation of the learner and cooperative methodologies for learning, is the most appropriate to develop educative programs through the web with electronic devices (Candy et al., 1994; Inglis et al., 1999; Knapper and Cropley, 2000; Sampedro et al., 2001).

As well as the pedagogical approach, the pedagogical design of the courses and the multimedia educative materials characteristics shall be considered.

The *pedagogical design* consist of a plan in which all the elements contained in the teaching-learning process must be structured in an appropriate way. The quality of this plan will led to the quality of learning and its products. In short, the elements included in a pedagogical design are the following:

Training Needs Objectives Contents Activities Methodology
Timetable Resources **Multimedia educative materials** Evaluation

Multimedia educative materials (MEMs) are essential resources for eLearning. These materials must be designed with high pedagogical and technological quality if they want to be useful in these arena. That is the reason why a multidisciplinar developmental team composed of pedagogists, informatics, graphic designers and experts in the content to be learnt is needed to produce good MEMs.

TRAINING OF DESIGNERS, TRAINERS AND STUDENTS

The training component of eLearning is crucial if we take into account the initial stage of development in which formative processes through the web are involved. In fact, the eLearning initiative and action plan promoted by the European Commision come as a result of the need to learn about how all the factors involved in learning through the web operate so that training of the main agents involved can be organized. Among these agents, the different training needs of MEMs designers, trainers and students must be considered (Inglis et al, 1999, Collison, 2000; Horton, 2000). It is important to remember that the quality of learning depends on a great deal of the quality with which these agents put into practice their respectives roles.

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SP9 - SEMIAUTOMATED METHOD TO QUANTITATE JOHNSEN SCORE ON TESTIS BIOPSIES BY IMAGE ANALYSIS

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Austria

STUDY OBJECTIVE

To investigate the possibility of creating an image analysis routine using multiple nuclear texture features to automatically determine a Johnsen Score equivalent of testis biopsies.

DESIGN

The study population consisted of 144 samples (96 preparations from orchiectomies, 27 specimens of tumour surrounding non-pathological testis-tissue and 21 samples from autopsies). Nuclear texture features were determined using image analysis of single-cell preparations. Discriminant functions were analyzed by multivariate regression analysis in order to build a binary tree for distinguishing cell maturation. A training-set was computed according to multiple regression which facilitated the computing of a Johnsen-score equivalent. The relation was proven by a Pearson‘s correlation test.

RESULTS

Due to regression analysis of the training set we could attain a general straight line equation which is based on the partial regression coefficients and the percentages of the single cell types. The analysis of the test-set according to that scheme showed a statistically significant correlation between the common semiquantitative method and the calculated Johnsen-score equivalent (Pearson 0,747 p=0,01). The average difference between both techniques was 0,98 (sd=0,799).

CONCLUSION

DNA image analysis using nuclear texture features revealed to be a suitable tool to evaluate the fertility of patients using single cell preparations of testis biopsies.
The characteristics of this method ensure a high degree of accuracy and a maximum potential of automation.

SP10 - HAEMATOLOGICAL CYTOLOGY IMAGE ANALYSIS AND SEMANTIC INDEXING: TOWARDS A GLOBAL APPROACH

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We present the architecture of a software system which yields an integrated platform for the morphological image analysis, pattern recognition and visual content-based indexing of peripheral blood smear images. This image analysis approach, based on tools of mathematical morphology, provides an objective description of blood cells and, more specifically, the system is designed to assist pathologists -it is not an automatic cell classifier- to diagnose lymphoproliferative disorders. A first preprocessing module implements the automated detection of working area of the blood smear, in images scanned under low magnifying power. When the best area is detected, the magnifying power is increased (x100) and the cell images are digitized and processed.

The cell analysis is performed in three steps: segmentation, feature extraction and classification. In our system, which has a high degree of content understanding, the image segmentation is organized in three modules. The goal of the first one is a fast segmentation of the initial blood image: erythrocyte segmentation (input to the erythrocyte feature extraction module) and leukocyte extraction (each leukocyte is extracted in a subimage). The leukocyte subimages are the input to the leukocyte classification module, which allows us the identification of lymphocytes. For every lymphocyte, a second module of segmentation decomposes the subimage into two meaningful regions: nucleus and cytoplasm. Additionally, a nucleus inner segmentation module is included in order to split the nucleus into chromatin regions having similar perceptual properties. Taking the nucleus and cytoplasm images as the starting point, the lymphocyte feature extraction module provides a global descriptor which contains the whole morphological information of each lymphocyte (shape, color and texture). Using this description, the lymphocyte classification module (a statistical classifier) assigns a lymphocyte population to one of the classes defined. When the set of descriptors and classification information are computed, the resulting description takes the form on an XML document (normative format in MPEG-7, very efficient for editing, searching, filtering and processing information in databases) using the indexing engine module. New applications, such as epidemiological databases, collaborative diagnosis, telehaematology, etc., can be developed adding networking abilities to the system.

SP11 - AUTOMATION IN CERVICAL CYTOLOGY

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The processes of preparation, screening and reporting of cervical smears are labour intensive and are highly dependent on a well trained and motivated staff. Even in the best run screening programmes, a small number of people will develop invasive cervical cancer in spite of having regular smears performed. A proportion of these are due to sampling errors and a proportion are due to laboratory false negatives. Any methods which have the potential to reduce these errors warrant careful evaluation.

Automated methods of cervical screening have been proposed for several decades but it has only been in the past 10 years that technological advances have made these a realistic objective. Automation can be applied for specimen preparation and for primary screening of slides.

Automated preparatory methods rely on placing the instrument used for taking the smear in a liquid medium (liquid based cytology), detaching the cells and making monolayer preparations. The two most widely used commercial systems are ThinPrep and AutoCyte measuring blood and polymorphs in the smear and allow optimum fixation of every specimen. Many studies have shown a reduction in the unsatisfactory rate and also a higher sensitivity for abnormalities. In the United Kingdom, several pilot schemes of liquid based cytology are currently in progress and the results will be available soon.

Automated screening devices rely on computer aided image analysis to detect abnormal cells although the ultimate diagnosis of abnormal smears depends on human interpretation. The two most widely used commercially produced devices use different approaches. One (Papnet) presents images of the most abnormal fields on a computer screen while the second (AutoPap) assigns an abnormality score to the slide. Slides with the lowest scores may not need human review. Papnet is no longer available although previously released machines are still in use. The recent addition of location guided screening to the AutoPap system allows the cytotech or pathologist to review the cells that have contributed to the abnormality score.

It is likely that automation will play an increasing role in cervical cytology in the future although full evaluation of the capabilities and cost-effectiveness of these new technologies is essential.

SP12 - GENE EXPRESSION STUDIES USING AFFYMETRIX GENECHIP AND SPOTTING TECHNOLOGIES, APPLICATIONS IN MOLECULAR DIAGNOSTICS.

Fiona Brew

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Micro Array technologies allow us to monitor the gene expression of many genes in one experiment giving us a global picture of gene expression and how it changes with different conditions. This has started to open up the discovery of genes and gene pathways involved in disease processes (Golub et al 1999 and Thykjaer et al 2001)

As this type of research progresses our ability to home in on particular diseases and the genes and pathways involved in their initiation and progress will increase enormously. With this knowledge it will then be possible to look closely at how drugs have their action and the reasons for the variation between individuals responses.
Once we have elucidated the genes whose expression levels are crucial in a disease process it will be possible to make arrays containing only detectors for these genes and so use gene expression in the diagnostic process.
Further to this it will also be possible to monitor the response of the patient to the prescribed drug, giving very early indications of the success or failure of a particular drug regime.

Micro array technology is likely to play a key role in the Health Management of the future using focused arrays containing a small number of genes which are highly validated markers for particular diseases along with arrays which may be able to elucidate a patients genotype and hence help to predict the best drug and dose to administer.

SP13 - MODERN ASPECTS OF TELEPATHOLOGY WITH SPECIFIC RELEVANCE TO ARTIFICIAL INTELLIGENCE (AI) SYSTEMS

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DEFINITION AND BACKGROUND

Telepathology is the diagnostic work of a pathologist at a distance. It has been developed to routine application in on-line application (frozen section service) and off-line use (expert consultation) within the last ten years. Both applications cannot replace modern molecular biological techniques which are of increasing importance in diagnostic pathology. The increasing use of telepathology and the existence of digitized information in pathology argues for implementation of artificial intelligence systems (AI) in application of telepathology.

THEORETICAL CONSIDERATIONS

Telepathology comprises the acquisition and transport of digitized images, the detection and extraction of information content, and its transformation into a diagnosis. Active (on-line) telepathology requires adequate stratified sampling procedures in order to detect the location and amount of interesting diagnostic information within a slide, passive (off-line) telepathology depends upon the quality and magnification of the transmitted images as well as on the correct selection of images displaying diagnosis-related information. Both applications need stratified sampling techniques and offer data sets which can be fed into AI systems such as neural networks, Bayesian decision models or non-hierarchic discriminant analysis. These models can be applied to search for “information-related” image descriptors, which then might serve as dynamic descriptors for selection of images, definition of appropriate image size and magnification in close association with the diagnoses under consideration.

FIRST TRIALS

Digital images of cytologic smears of pleural effusions and transbronchial fine needle biopsies were analyzed according to their gray value distribution prior to electronic transmission. Only those areas were selected for transmission which revealed significant gray value scores above the background threshold. In addition, the spatial gray value distribution was fed into a neural network (easy shell), and related to further image processing based upon syntactic structure analysis. Only those images were selected for final diagnostic evaluation which the hierarchic AI procedure permitted for electronic transfer.

RESULTS AND DISCUSSION

The screened area of slides could be reduced to 10% of the total slide. No significant information was lost. In addition, it could be shown, that AI can provide an accurate image transfer steering mechanism for accurate final diagnostic judgement. As final result, an AI system automatically limits the number of transferred images and their magnification. AI systems in telepathology can be used for assessment of a

dynamic quality control in diagnostic pathology, and efficient control of dynamic microscopes via the internet.

ORAL PRESENTATIONS

OP1 - EXPRESSION OF CLEAVED CYTOKERATIN-18, ACTIVE CASPASE-3 AND KI-67 IN RELATION TO GASTRIC MUCOSAL ATROPHY.

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INTRODUCTION

Gastric mucosal atrophy is defined as 'loss of specialized glands'. The role of apoptosis and proliferation in the development of gastric mucosal atrophy is unclear. It has been suggested that in mucosal atrophy the glands are not lost, but only pushed aside by inflammatory cells and edema. If the glands/epithelium are destroyed e.g. by inflammation, this is most likely to happen by apoptosis. During apoptosis of epithelial cells, cytokeratin-18 (CK18) is cleaved upon caspase-3 activation. Aim of the present study was to evaluate the expression of these proteins in normal gastric mucosa, gastritis, gastric mucosal atrophy and intestinal metaplasia (IM).

METHODS

Twenty-six resection specimens, consisting of 2 cases with normal mucosa, 15 with gastritis and 9 with IM were included and stained with the monoclonal antibodies M30 CytoDeath, anti-active caspase-3 and Mib-1. Frequency of immunopositive cells was evaluated and correlated with the respective gastric mucosal lesions.

RESULTS

Immunopositivity for cleaved cytokeratin-18 and active caspase-3 was detected at the surface epithelium and in the top of the gastric pits. Cleaved cytokeratin-18 was co-expressed with active caspase-3 in all cases. Mib-1 positivity was found in the neck region of gastric units in normal mucosa and gastritis, while in IM Mib-1 positivity was found in the base of the crypts. In resection specimens an increased apoptotic rate was found in gastritis and IM compared to normal mucosa.

CONCLUSIONS

These data support a role of a increased apoptosis/proliferation ratio in the pathogenesis of gastric mucosal atrophy and IM.

OP2 - REGRESSION OF GASTRIC MUCOSAL ATROPHY AFTER ERADICATION OF HELICOBACTER PYLORI.

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BACKGROUND

Grading gastric mucosal atrophy in antrum biopsy specimens remains a controversial subject, because of limitations in inter-observer agreement. We previously described a reliable, quantitative method for grading atrophy of the corpus mucosa with excellent reproducibility and good correlation with the Sydney scores. The aim of the present study was to evaluate the applicability of this method for the grading of antral atrophy.

METHOD

Antrum biopsy specimens were collected from 71 gastroesophageal reflux disease (GERD) subjects at baseline, and after 3 and 12 months. All subjects received omeprazole 40 mg o.d. after ther first endoscopy for 12 months. After randomization 27 of the 48 H.pylori-positive patients also received eradication therapy. In 182 HE stained specimens, the proportions of glands (VPGL), stroma (VPS), infiltrate (VPI) and intestinal metaplasia (VPIM) in the glandular zone of the antrum mucosa were measured as volume percentages using a point counting method. In these specimens mucosal atrophy was assessed by 2 experienced GI pathologists (EB, JL) according to the updated Sydney classification as either non-atrophic mucosa (n=47), or as mild (n=29), moderate (n=50) or marked (n=56) atrophy. In addition, a group of 23 cases with difficult to classify grades of atrophy were included.

RESULTS

The mean VPGL decreased with increasing Sydney grades of atrophy ($P<0.001$), while the mean VPS and VPI increased (both $P<0.001$). After H.pylori-eradication even the cases with lowest VPGL regressed to normal levels.

CONCLUSION

Overall, a low VPGL is correlated with antrum mucosal atrophy. The present data indicate that gastric mucosal atrophy is reversible, since almost all cases showed regression of VPGL after H.pylori-eradication. The cases with difficult to classify grades of atrophy showed significantly lower VPGLs and higher VPIs than the other cases.

OP3 - MICROARRAY COMPARATIVE GENOMIC HYBRIDIZATION REVEALS A NARROW GAIN AT 20Q13 IN HUMAN GASTRIC CARCINOMAS.

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INTRODUCTION

As detected by comparative genomic hybridization (CGH), gain of chromosome 20 is a frequent aberration in gastric carcinomas (Van Grieken, J Pathol, 2000). In order to investigate this chromosome with a higher resolution and sensitivity, we performed microarray CGH (both a scanning and a high-resolution array) in a series of 27 gastric cancers.

MATERIAL & METHODS

The scanning and high-resolution arrays have been described previously (Pinkel 1998, Albertson 2001). The scanning array is comprised of 27 clones, and the high-resolution array contained 27 clones at 20q13.2. The high-resolution array was used to narrow down the amplicon at 20q13.2 in the 3 tumors that showed amplification of this chromosomal region with the scanning array. The integrated tumor to reference fluorescence ratio was calculated per spot. Thresholds of 0.8 and 1.2 were used for losses and gains, respectively. To confirm the microarray CGH data, FISH has been performed on selected cases for 20q13.

RESULTS

Gains on chromosome 20q were detected in 12 of 27 cases (44%). These changes included gains of the whole arm of chromosome 20q in 8/27 (30%) of the cases, a gain restricted to 20q12.1 in 1 case, and gains restricted to 20q13 in an additional 3 cases. FISH experiments showed a copy number increase for 20q13 in all 3 tumors harbouring 20q13 amplification as detected by microarray CGH. The 3 tumors that showed amplification of chromsomal band 20q13 were investigated in more detail with a high-resolution array (contig-array for 20q13.2). In one tumor the whole length of the contig was amplified at a constant level. In the other two tumors a clear breakpoint was found within the 20q13.2 region. The region in between these 2 breakpoints is ~800 kb.

CONCLUSIONS

In the present study we demonstrate, with microarray CGH, that the amplicon on 20q13.2, harbouring one or more putative oncogenes relevant to gastric cancer, can be narrowed down to 800 kb.

OP4 - PROGNOSTIC SIGNIFICANCE OF REGIONAL LYMPH NODE QUANTITATIVE HISTOPATHOLOGY IN GASTRIC CARCINOMAS

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INTRODUCTION

The international scientific studies of standardized tumor classification and prognostic factors have the effect of a recent TNM system from 1997, that takes into account the number of involved perigastric lymph nodes, having a major role in quality management in medicine. The aim of this study is to apreciate the histologic appearance of the first draining lymph nodes, correlated with localization and histologic feature of primary tumor, and to estimate quantitative criteria which could be used as guidelines for prognosis.

MATERIAL AND METHODS

The authors have studied 33 gastric carcinomas, surgically obtained in Emergency Hospital from Iassy. The age of the patients was comprised between 22 and 75 years. The pieces were processed through paraffin-technique and stained with H&E, and examined for general appearance and quantitative histologic parameters. The nuclear morphometry, mitotic activity index and stereological measurements were made on 4 μm histological sections, the most differentiated and atypical portions of the primary tumor and nodes being selected. It was used a professional program and we quantified the volume percentages of tumoral cells in primitive tumor and metastatic cells in nodes, results being calculated automatically.

RESULTS

Pathological examination of the primary tumor, which was removed by total or partial gastrectomy with histologically tumor free-margins, shows three forms of gastric tumors: adenocarcinoma, colloid and signet ring cell carcinomas. 57.57 % of cases have metastases in regional perigastric lymph nodes, along the lesser and greater curvatures and the nodes along the left gastric, common hepatic, hepatoduodenal, splenic, and coeliac arteries, according to primitive tumor localization. Distant metastasis in other intra-abdominal lymph nodes such as retropancreatic, mesenteric and para-aortic we have not identified in these cases. The microscopic forms of lymph node metastases were similar with the primitive tumor: adenocarcinoma (68.42 %), colloid (5.26 %) and signet ring cell carcinomas (26.32 %).

CONCLUSIONS

The nuclear parameters (area, perimeter, and volume) have a noteworthy prognostic factor exceeding the value of mitotic activity index, in each histologic type. The stereologic measurements of the first draining node showed a close relation with survival rate.

OP5 - CORRELATION BETWEEN CHROMOSOMAL INSTABILITY AND CHROMATIN PATTERN ABNORMALITIES IN COLORECTAL ADENOMAS AND CARCINOMAS

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BACKGROUND

Colorectal cancer, like all cancers, is caused by a disruption of vital biological processes due to genetic alterations. Changes in chromatin patterns, detectable by DNA texture analysis, are believed to be among the earliest detectable morphologic alterations which reflect these genetic changes. In colorectal cancer, a major part of these genetic alterations occurs at the chromosomal level, either as numerical changes or as structural rearrangements. It therefore seems plausible that the presence of chromosomal aberrations is related to changes in chromatin patterns.

AIM

To test the hypothesis that changes in chromatin patterns measured by DNA texture analysis are correlated to the chromosomal aberrations in colorectal adenomas and carcinomas as detected by comparative genomic hybridization (CGH).

MATERIAL AND METHODS

In a series of 8 adenomas and 21 carcinomas, DNA texture analysis and CGH were performed. From these cases, a total of 574 adenoma nuclei and 1,741 carcinoma nuclei were analysed for nuclear texture.

RESULTS

Two adenomas (25%) and 10 carcinomas (48%) showed a high level of chromosomal instability (HL-CIN), while 6 adenomas (75%) and 11 carcinomas (52%) showed a low level of chromosomal instability (LL-CIN). In the adenoma group, many DNA texture features showed significant differences between HL-CIN and LL-CIN, including optical density parameters (e.g. MAXOD p=0.000, SKEWOD p=0.000), area parameters (AMHIGH p=0.01), run length features (e.g. RLNU0 p=0.000) as well as several other parameters (e.g. ENTROPY p=0.000, CONTRAST p=0.000, HOMOGEN p=0.000). In the carcinoma group, only 3 variables differed between the LL-CIN and HL-CIN group (MAXOD p=0.000, DENLS p=0.000, and DENDS p=0.000).

CONCLUSIONS

In this small series of tumours, chromosomal aberrations in colorectal adenomas, as detected by CGH, are clearly related to abnormal chromatin patterns as detected by DNA texture analysis. In colorectal carcinomas, this relationship was virtually absent except for a small features relating to the density of the stained nucleus. Colorectal carcinomas that show only chromosomal abnormalities, must have gained the multiple genetic changes necessary for becoming a cancer in another way, which is also associated with changes in chromatin patterns.

OP6 - CGH ANALYSIS OF CLONALITY OF SYNCHRONOUS AND METACHRONOUS COLORECTAL TUMORS.

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It is still unclear wether synchronous and metachronous multiple colorectal tumors arise from a monoclonal or multicentric origin. To investigate this question on the chromosomal level, we performed genetic analyses on tumors of in total 33 patients: 5 with multiple adenomas (A+A), 24 with adenoma next to carcinoma (A+C), and 4 with multiple carcinomas (C+C), making a total of 109 tumors. In all instances, tumors within one patient were collected from the same region of the large bowel. Tumor DNA was obtained from microdissected paraffin embedded tissues and used for CGH and APC mutation analysis. After CGH analysis, 1 A+A and 5 A+C patients were excluded from analysis because the adenomas showed no chromosomal abnormalities, making a comparison with their neighbouring carcinomas impossible.

The remaining 27 could be divided in two groups: one of 17 patients, with neighbouring tumors showing very different gains and losses, and of 10 patients, with either identical or very similar patterns of chromosmal aberrations. It would seem that, while the majority of multiple tumors (17 of 23) appear to have arisen multicentrically, at least in a subgroup (10 of 23) seems to have a clonal origin. However, in 5 of the cases of the altter group APC mutations were found, which differed among the multiple tumors. Since APC mutation is considered a very early event in tumorigensis, this suggests that probably also these tumors originated independently.

In conclusion, our data support the idea that synchronous and metachronous colorectal tumors are genetically different and therefore multicentric in origin.

OP7 - KARYOMETRIC ANALYSIS OF PRECANCEROUS LESIONS IN COLORECTAL MUCOSA

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BACKGROUND

Colorectal carcinoma has a worldwide distribution, with the highest death rates in the United States and Eastern European countries. The development of carcinoma from adenomatous lesions is referred to as the adenoma-carcinoma sequence and is well documented, but pathologic diagnosis of early precancerous changes in colorectal mucosa is not well standardized.
Aims. To estimate sensitivity and specificity of karyometric method for early detection of colorectal carcinoma.

METHODS

Endoscopic biopsies of colonic mucosa surrounding carcinoma (n=42) and chronic colitis (n=25) were analyzed. After standard fixation and paraffin embedding, 4 μm thick sections were stained with hematoxilin and eosin. The mean volume-weighted nuclear volume of epithelial cells was estimated by point-sampled nuclear intercept method, by original test system, using objective 100x and total magnification of 1200x. Nuclear area, perimeter, circularity and integrated optical density were estimated by image analyzer, using objective 40 (N.A.= 0.65). In each case a hundred nuclei were measured. Nuclear volume value over 100 μm^3 and nuclear area over 20 μm^2 were considered as positive findings.

RESULTS

The best results were obtained with the mean volume-weighted nuclear volume; sensitivity and specificity were 97.4% and 92.7%, respectively.
Conclusions. Achieved results indicate that karyometric method can be used for early detection of precancerous changes in colonic mucosa.

KEYWORDS
colorectal carcinoma, precancerous lesions, karyometry, nuclear volume.

OP8 - IMAGE ANALYSIS FOR COMPARISON OF STROMAL TISSUE COMPONENT AT THE CENTRE AND PERIPHERY OF PANCREATIC TUMOURS

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OBJECTIVE

Pancreatic carcinoma is frequently associated with a marked desmoplastic response, but this stromal reaction has not previously been used as a prognostic factor because of the impracticality of obtaining quantitative measures using manual visual analysis. In this paper we use computer image analysis techniques to measure the change in fraction of stromal tissue between the centre and periphery of pancreatic tumours.

STUDY DESIGN

Nineteen cases of pancreatic carcinoma treated by pancreaticduodenectomy were studied. Tissue was received fresh and fixed in formalin for 48 hours. The head of the pancreas was serially sliced parallel to the common bile duct and blocks of the tumour were taken which included surgical resection margins. Blocks retrieved for this study were stained using the sirius red-light green method. Five images from the centre and five from the periphery of each tumour were digitally captured using a 4x objective (8.64 mm^2 per frame). An image segmentation technique based on cluster analysis of colour content was used to measure the fractional area of stroma. Repeatability of the image analysis technique was established previously by comparison with manual point counting.

RESULTS

In 9 samples, the fraction of stroma was significantly greater at the margin than at the centre of the tumour ($P < 0.05$) with a mean increase in stromal fraction of 17.6 9.4 percentage points. None of the remaining cases had significantly more stromal tissue at the centre than at the margin.

CONCLUSIONS

The computerised image analysis technique used in this study permits the fraction of stromal tissue in images of pancreatic carcinoma to be measured rapidly and accurately. This facilitates studies of spatial tumour composition which would be impractical with manual methods.

OP9 - CHROMOSOMAL ABNORMALITIES AND THEIR PROGNOSTIC VALUE IN 270 DE NOVO ACUTE MYELOID LEUKEMIA

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The aim of the study was to determine the predictive value of karyotype in 270 patients diagnosed with de novo AML between 1985 and 2000
The mean age was 50.4 (range 1-89).There were 158 men and 112 women. According to the FAB classification, there were 15 M0, 44 M1, 62 M2, 57 M3 (including 14 M3v), 31 M4 (8 of them M4Eo), 46 M5 (including 4 M5 with erythrophagocytosis), 13 M6, and 2 M7. Trilineage myelodysplasia (TMDS) was present in 31 patients (11.5 %). 168/270 (58.5%) showed clonal karyotypic abnormalities. In the good prognosis(GP) group there were 11 t (8;21)/ 62 M2 (17.7%); 50 t(15;17)/57 M3 (87.7%). Cytogenetic abnormalities associated with intermediate prognosis (IP) include 7 inv h (16)/ 8 M4Eo (87.5%), 13 cases with trisomy 8 and 112 patients with normal karyotype. In the poor prognosis (PP) 5 trisomy 21, 5 trisomy 11 and miscellaneous (+4, +6,+12,+17,-21,-20,
t(8;16), t(9;11), add 17, inv11, del X (q13), del 7p). In the worse prognosis (WP) group we included six 3q21q26 rearrangements, 2 t(9;22), 6 rearrangement of chromosomes 5 or 7 and twenty-eight patients with complex karyotype. From 223 patients treated intensively, 163 (73%) achieved CR. The median CCR of patients who achieved CR was 11.6 months and it significantly varied between the cytogenetic categories (p=0.00001). GP group had the longest CCR (mean 113 months; median, not attained) followed by IP (median 11,6, mean 37,6 months), PP (median 3.7, mean 19.8 months) and WP (median 0, mean 2,9 months).CCR was longer in young patients (p=0.038) with FAB subtype M3 (p=0.0001), and who received BMT(p=0.00001) but only in patients with normal karyotype or good prognostic abnormalities, consolidation was with BMT. The median OS of all patiens was 9.7 months and 24.5% of patients were alive at 3 years. The median and mean of OS was: GP (median 29.1, mean 89.7 months), IP (median 12.7,mean 33.5 months), PP (median 5, mean 17.8 months) and WP (median 2, mean 4.6 months), with significant difference among groups (p=0.00001). Patients under 60 years (p=0.0002) and who received BTM (p=0.00001) had a longer overall survival but this was significantly shorter in patients who had TMDS (p=0.0005) This was found more frequent in complex karyotypes. Multivariante analysis showed that only karyotype and treatment are independent prognostic factors for CCR and OS.

CONCLUSIONS
In our study, like others, the best prognosis was for young patients, with t(8;21) or t(15;17), without TMDS and who received consolidation BMT. However, older patients with TMDS and complex karyotypes, had a poor outcome. Normal karyotype, inv h (16) and trisomy 8 presented an intermediate prognosis. The cytogenetics (morphologic and molecular) permit now desing theraphy risk-adapted.

OP10 - IMAGE PROCESSING AND ANALYSIS SYSTEM IMAGEWARP

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ImageWarp is universal image editing, processing and analysis software, which combines the power of graphic development environment, comprehensive image analysis toolset, and programming development techniques. Designed to work under Windows 98/ME and NT/2000, *ImageWarp* offers full compatibility with standard capture hardware, hundreds of high-performance image processing and measurements functions, built-in automatic script language, databases and spreadsheets support, and various graphic representation of output data. Unlike any other image analysis software, *ImageWarp* incorporates graphic editing tools and functions that work with all known image types including 16-bit, 32-bit and 64-bit (complex) images. A multiple window interface allows the simultaneous display of images, charts, and measurements for the rapid creation of custom applications and development of new imaging techniques. *ImageWarp* employs a multithreading processing engine, which provides parallel execution of several functions at the same time. Most of the processing functions are optimized for Intel MMX technology. Automatic and interactive measurement functions support more than 50 built-in and user-defined geometrical and optical parameters.

A number of proprietary processing algorithms and interface innovations make *ImageWarp* especially useful for the tasks of quantitative morphology, cytology and bioinformatics. Ability to utilize gray scale maps and palettes for 16-bit monochrome images makes it a perfect tool for viewing and manipulating genomic microarrays. A comprehensive set of color processing algorithms – from multiphase color segmentation to mathematical morphology operations in RGB, HLS and YIQ coordinate systems – proved to be very useful in live cell research and immuno-fluorescence.

One of the recent applications of *ImageWarp* involved morphometric investigation of neuroprotective action of Diavitol on glial cells culture. The action of the medication was examined by means of the analysis of the intensity of the neurotransmitter neuronal capture in the cerebrum regular centers of laboratory animals and by using neuroglia cell cultures under the conditions of hypoxia

Detailed information about *ImageWarp* software could be found at www.imagewarp.com

OP11 - A MODULAR NEURAL NETWORK SYSTEM FOR THE ANALYSIS OF IMMUNOSTAINED NUCLEI IN HISTOPATHOLOGICAL SECTIONS

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The evaluation of immunocytochemically stained histopathological sections presents a complex problem due to many variations that are inherent in the methodology. This communication describes a modular neural network system, which is being used for the detection and classification of breast cancer nuclei, named Biopsy Analysis Support System (BASS). The system is based on a modular architecture where the detection and classification stages are independent. Two different methods for the detection of nuclei are being used: the one approach is based on a feed forward neural network (FNN) which uses a block-based singular value decomposition (SVD) of the image, to signal the likelihood of occurrence of nuclei. The other approach consists of a combination of a receptive field filter and a squashing function (RFS). The classification module of the system is based on a radial basis function neural network. A total of 57 images captured from 41 breast cancer biopsy slides containing over 8300 nuclei were individually and independently marked by two experts. The nuclei were immunostained using specific antibodies against steroid recepotrs. A five scale grading system, known as diagnostic index, was used to classify the nuclei staining intensities. The experts' mutual detection sensitivity (SS) and positive predictive value (PPV) were found to be 79% and 77% respectively. The overall joint performance of the FNN and RFS modules were 55% for SS and 82% for PPV. The classification module correctly classified 76% of all nuclei in an independent validation set containing 25 images**.** In conclusion, this study shows that the BASS system simulates the detection and grading strategies of human experts. This system enables semiquantitative measurements to be performed on immunohistochemically stained nuclei in histopathological sections. In this context BASS has the potential to improve assessment accuracy and standardization procedures, which are much needed in histopathology.

OP12 - A NEW METHOD AND APPLICATION ON THE MEASUREMENT OF 3D BARRIERS THICKNESS FROM 2D SECTIONS

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Barriers is a layer structure which separate two spaces. The pathological changes of diseases often show some extent changes in certain barriers. So it is important to measure the barriers thickness. 3D barriers thickness is real barriers thickness. The methods to measure 3D barriers thickness based on the count of points and intersections, on the measurement of intercept have given. Although these methods are easy to apply, it can be hardly used for image analysis system.

AIM

To measure 3D barriers thickness from 2D sections by image analysis system, the study was done.

MATERIAL AND METHOD

Formulae to measure 3D barriers thickness ( ) from 2D sections was deduced based on the principles and methods of stereology and image analysis. Based on the methods of points and intersections count and on the apparent width measurement, the relative errors by our method were analyzed. And then our method was used to measure the thickness of glandular epithelia of colorectal adenoma and carcinoma.

RESULTS

The setup formula to calculate is as follow: $\sum A_b / (4\sum B_b) = \sum A_b / [2 \sum B_{b1} + B_{b2}]$. In the formula, A_b and B_b are separately the area and one side length or boundary of the studied barriers in 2D sections. B_{b1} and B_{b2} are separately the two sides lengths or boundaries of barriers in 2D sections. The relative errors are 7.18% and 2.19% when comparing separately with the method of points and intersections count and with the method of apparent width measurement. The barriers thickness by our method is not affected by the orientation of measurement. Applying our method to measure the epithelia thickness of glands of colorectal carcinoma, adenoma and normal, we found that the thickness of well differentiation colorectal carcinoma is significantly smaller than that of goblet cellular adenoma, columnar cellular adenoma, mixed cellular adenoma and normal large bowel glandular epithelia, $p<0.05$. The thickness of goblet cellular adenoma is significantly higher than that of normal large bowel glandular epithelia, $p<0.05$.

CONCLUSION

The formula shows that 3D barriers thickness can be well acquired based on the measurements of area and lengths or boundaries of barriers by computer image analysis from 2D sections of barriers structures by our method. It is easy to apply, and it is useful in quantitative describe the thickness of glandular epithelia of colorectal adenoma and carcinoma.

KEYWORDS

Barriers Thickness, Stereology, Image Analysis, Colorectal Carcinoma

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OP13 - CELL STRUCTURE IDENTIFICATION METHOD

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The morphometric analysis of a cell involves defining such parameters as area, diameter, cell-cytoplasmic ratio.
The area is defined as the sum of all of the object pixels with the exception of the boundary ones. The geometric measurements involve three kinds of boundary pixels that contribute to the area and perimeter values:

- Vertical; the contribution corresponds to the scale value of a pixel.
- Horizontal; the contribution corresponds to the scale value of a pixel.
- Diagonal; the contribution corresponds to the $\sqrt{2}$ of the scale value of a pixel.

As a result:

$$AREA = \sum_{object} \begin{cases} \sqrt{2} \cdot unit, & for\ diagonal\ pixels \\ unit, & otherwise \end{cases}$$

where AREA is the cell area, and unit is the measurement unit.
And diameter:

$$D = \sqrt{\frac{4 \cdot AREA}{p}}$$

In order to obtain the topological characteristics, it is convenient to represent a cell as a hierarchy of binary images where the image of the cell lies in the root of graph, on the next level - the nucleolus and various cellular impurities lie in the branches.

The hierarchy principle can be implemented in computing by using bits.
It is optimal to single out eight hierarchy levels because in computing a pixel is most often characterized with the help of bytes, and each byte is equal to eight bits. One multi-phase image allows to draw conclusions on all of the classification levels. If there is a nucleus in a cell, the pixel value of this point will be 3. If there is a nucleolus in the nucleus, the pixel will be equal to 7. In case there is cellular impurity outside the nucleus, the value of pixel will be 5. The optimal condition for pixel P of a theoretical cell is the following:

(P **AND** 1) **OR** (P **AND** 3) **OR** (P **AND** 7),

This means that a theoretical cell contains a nucleus, and the nucleus contains nucleoli.

OP14 - AN EASY WAY TO DETERMINE THE THICKNESS OF PARAFFIN SECTIONS

Mairinger T, Tauber M, Gschwendtner A

The exact knowledge of the thickness of the paraffin sections under investigation would be of great interest for many types of image analysis. The methods proposed so far by several working groups have proven to work reliably, but include time-consuming preparation techniques.
This paper describes the evolution of the method published by the authors in 1995. Originally the section was divided into 2 parts, one was reembedded, oriented orthogonally to its original plane and resectioned through its original thickness. The critical step of the formerly described procedure was the reembedding of the section and its reorientation.
The method presented in this paper is also based on conventional paraffin sections.. After sectioning, the tissue section is not put on a glass slide, but on the coverslip glass. This is done in such a way that the one small part of the section is wrapped around the edge of the coverslip glass. The paraffin section is then processed and stained on the coverslip glass without the conventional glass slide.
After staining, the specimen is put orthogonally in a tube filled with Glycerol-Gelatine. The diffraction index of this fluid is similar to that of microscope oil, which allows the use of conventional oil immersion objectives. When focussing on the edge of the coverslip glass, the section can be seen in its full thickness, wrapped around the glass edge. The thickness of the section can easily be measured by using any length measurement device available in light microscopy.

OP15 - A NOVEL SCANNING IMAGE CYTOMETER FOR AUTOMATED INTERPHASE FISH ANALYSIS

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MetaSystems GmbH

Current interphase FISH analysis is tedious and subjective, particularly if more than 2 fluorochromes are used for labeling. Automated scoring would be highly desirable to extend the number of analyzed cells, to use objective scoring criteria, and to document the investigated cells.

We developed an automated systems for interphase FISH scoring. MetaCyte scans slides to detect analyzable areas . Depending on the application these may include isolated individual cells or clusters of a certain cell type. Images are then captured from different focal planes and combined to an extended focus multi-color image. FISH signals are identified, counted, and their x-y-z-positions are recorded. The system provides spot count and detects co-localisations of differently labeled probes. During the scan a cell gallery is created for subsequent on-screen review of the results. If necessary, any individual cell can be automatically relocated under the microscope for direct visual inspection.

As various morphometric and intensity features can be measured during the scan FISH results can be correlated with shape information and DNA content.

Due to its high speed of up to 100 cells per minute the system is particularly suited for extending the detection limit of rare events.

We will introduce the design concepts of the system and present results for several FISH applications.

OP16 - QUANTITATIVE ASSESSMENT OF PROLIFERATIVE ACTIVITY IN BORDELINE CHONDROSARCOMA OF BONES

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INTRODUCTION

Chondrosarcoma (CS) are three histologic grades, but segregation of grade I tumors from chondromas can be difficult. The aim of this study was to assess the quantitative parameters of bordeline CS of bone and to estimate quantitative criteria, which could be used as guidelines for risk grade, prognosis and treatment.

MATERIAL AND METHODS

We conducted a clinicopathological analysis of chondroma and CS in 12 patients surgically treated and histopathologically diagnosed. The CS were classified into three grades of malignancy according to Evans' microscopical classification and further divided into low and high grades of malignancy. The quantitative standard measurements of tumoral cells (area, diameter, perimeter, long axis, short axis, etc), stereology and proliferative activity (mitoses/mm^2, mitotic index) assessment were made on the representative sections. Our quantitative results were statistical analized to determine wheather they correlated with tumor behavior. Immunohistochimical study used cellular proliferative markers as PCNA and P53 correlated with histological grades. We quantified the number of positive and negative cells after an immunohistochemical technic on fixed paraffin-embedded tissue.

RESULTS

The nuclear area was larger in high-grade CS and significant differences were found betveen chondroma and CS. The percentage volume of tumor cell nuclei was significantly increased in high-grade CS than in low-grade CS or chondroma. The mitotic figures are rarely, but increased when compared with chondroma. The percentage of PCNA-immunoreactive nuclei was low in tumoral cells and did not reveal significant differences between benign, low- and high-grade tumours.

CONCLUSIONS

Quantitative microscopy is helpful through the demonstration of the invasive activity in relation to the host bone. The measurement of nuclear parameters and proliferative activity appears to offer a method of differentiation of benign from low-grade from high-grade tumors.

OP17 - QUANTITATIVE ANALYSIS OF THE MICROVASCULAR DAMAGE OF THE MYOCARDIUM IN SEPSIS

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INTRODUCTION

Previous experimental studies have shown that the microvascular dysfunction which occurs in sepsis involves all three elements of the microcirculation: arterioles, capillaries, and venules. The objective of the present study was to define the relationship between the coronary alterations and the cardiac dysfunction in sepsis.

MATERIAL AND METHODS

We have studied fourty-eight patients, who died of sepsis caused by abdominal infections sepsis and were been postmortem examination. The fragments from coronary arteries and the myocardium from all cavities of the heart have been processed by paraffin technique. The slides have been examined for qualitative diagnosis. The quantitative study has been done with a digital interactive program, which utilized the stereology of procentual volumes of myocardial components (muscle cells, vessels and connective tissue), all results being compared with hearts witness.

RESULTS

Autopsy studies provide circumstantial evidence that histology changes are also a feature of myocardial injury in human sepsis. The number of perfused capillaries of the myocardium are reduced, in the venules is an inflammatory response characterized by neutrophil infiltration and PMN-endothelial adhesive interactions. We noted alterations in the myocardium that consisted of interstitial miocarditis (29.16%), interstitial edema (25%), and muscle-fibres necrosis (10.41%). We remarked differences among heart cavities, vessels of the ventricles being more affected. Morfometry relieved the increased of interstitial space and decreased of vascular lumina.

CONCLUSIONS

The present study suggests that objective coronary microcirculatory damage and myocardial cell injury associated with sepsis, thereby impacting on oxygen diffusion to mitochondria.

OP18 - MORPHOMETRIC EVALUATION OF AGNOR IN SOFT TISSUE SARCOMAS

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Nucleolar organizer regions (NOR) are intranuclear structures that are seen as fibril centers at electron microscopy. These centers are visible in interphase nuclei when paraffin-embedded histologic preparations are examined after a silver staining. NOR identified by means of an argyrophilic technique (AgNOR) were shown to be of value in prognosis of malignant tumors. Due to its sharp optical contrast, AgNOR can be easily quantified by morphometric procedures and seems to be a convenient way to study routine histologic preparations to assess tumor proliferation rate.
In our study, we have quantified: the number of nucleoli/field; the number of AgNOR/field. For each of these two, we have followed the area, perimeter and the color intensity. Our results indicate that AgNOR expression has a significant prognostic role. Tumours at lower stages have a lower expression of AgNOR than those with more advanced disease. Tumours with high histopathologic grade have a higher expression of AgNOR.

OP19 - SPECTRAL MORPHOMETRIC CHARACTERISATION OF CHROMATIN CONDENSATION IN PROSTATE NUCLEI

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The quantitative examination of prostate histology offers new clues in the diagnostic classification of prostatic lesions. The aim of this study was to examine the use of Multi-spectral imaging (MSI) techniques in the characterisation of chromatin condensation in prostate nuclei.

The spectral characteristics of standard haematoxylin and eosin stained prostate specimens were evaluated using light microscopy and a liquid crystal tunable filter (LCTF) based spectral imaging system. Images were recorded across the visible spectrum 400-720nm (65 bands, 5 nm intervals) forming a multi-spectral "data cube". Regions exhibiting benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia (PIN) and cancer (PCa) were imaged in ERDAS (.lan) format from which 58 nuclei were identified as exhibiting BPH (n=28), PIN (n=20) and PCa (n=21).

A K-Means unsupervised classification was applied to a sub-set of 11 BPH nuclei to identify 3 characteristic inter-nuclear regions of low, moderate and high chromatin condensation. For each region the average spectral profile was generated and a spectral reference library was created.

The localisation of differing inter-nuclear spectral components was achieved using the Spectral Angle Mapping (SAM). This is a physically based spectral classification that uses the n-dimensional angle to match pixels to reference spectra. The methodology has the added advantage for light microscopy in that it is relatively insensitive to varying illumination. For each nucleus the area percentage of each spectral component was measured together with the number of occurrences. Results indicate that using these spectrally defined features there was few significant differences between BPH and PCa. Comparison of BPH v's PIN and PIN v's PCa showed the area percentage of low chromatin condensation to be the strongest feature, giving up to 97.6% correct classification. PIN seems to form a separate morphological group with an increased amount of low chromatin condensation. A 3-way discriminant function was capable of correctly classifying 77.6% of all nuclei.

Distinct spectra characterise different extents of chromatin condensation in an objective and visual manner. Consequently, it is believed that spectral analysis could be used as an adjunct to conventional Morphometric methods.

OP20 - A QUANTITATIVE AND QUALITATIVE ANALYSIS OF FIBROSIS IN RESTRICTIVE CARDIOMYOPATHY

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BACKGROUND

Cardiomyopathies are heart muscle disease characterised by myocardial dysfunction, myocytes loss and fibrous replacement which can be reactive or reparative. This definition can be applied to all the idiophatic forms recently classified by the WHO/ISFC into dilated, hypertrophic, restrictive, arrhythmogenic right ventricular.

Quantitation of fibrosis can be made through different histochemical and immunohistochemical stains. Aim of our study was to test different histological techniques, currently used worldwide, and to compare them.

MATERIAL AND METHODS

We examined 600 sections from 9 patients affected by restrictive cardiomyopathy that beyond the well known myocytes disarray is also characterised by endo-perimisial and interstitial fibrosis. Three blocks were taken from the left, right ventricular free wall and interventricular septum. Five micron thick sections were cut and stained with Haematoxylin-Eosin, Sirius red and Heidenhain trichrome stain. To quantitate the interstitial collagen extension we used a light microscope coupled with a 3CCD video collagen type we add to the microscope polarised lens.

Results: the mean percent collagen content in the myocardium was 20.1±12 with the Heidenhain trichrome stain, whereas was 24.4±14.3 with the sirius red stain.

The mean percent amount of type I collagen was 77.24±4.6, range 68.8-84.7; the type III collagen was 22.8±4.6, range 15.3-22.8.

CONCLUSIONS

Quantitative evaluation of the myocardium collagen content revealed that the two different staining are not completely equivalent. Sirius red is able to detect a larger amount of the extracellular matrix component whereas Heidenhain trichrome stain detects more selectively only collagen fibres. For this reason the percent value with Sirius red is higher than with the Heidenhain trichrome. The linear regression analysis between these two staining methods showed a highly significant correlation ($p=0.0007$), thus quantitative values reported in literature for fibrosis should be corrected according to the different stain adopted.

OP21 - CARDIOMYOBLASTS IMPLANTATION (FIRST RESULTS) .

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Recently cardiomyoplasty by cardiomyocytes was successfully tried (Li K.R. et al., 1996 ; Watanabe E et al., 1998 ; Scorsin M. et al.,1996, Hagège A. et al, 1998 and other authors). This method is linked with cell culture which is expensive in spite of its advantages. Taking on account the existing experience of foetal organ implantation and ectopic growth, the aim of the present study was to check the possibility of foetal heart implant use for repair of heart injury or supply of myocardial insufficiency.

MATERIAL AND METHODS

35 Fischer rats, males and females were used as recipients. Heart from foeti aged 15-20 days i.u. development were implanted into a subcutaneous ear pouch of adult animals (with respect of animal bioethic rules). Biomicroscopy, biopsies with optic and electron microscopy, histochemistry were performed at different postoperative /p.o./delays. Seric IGF-1 was determined in recipients. Echography of the implant site, electrocardiography /ECG/ of both recipients and implants with adrenaline or vagal stimulation or without them were performed. Observation delays varied from 0 to 410 days.

RESULTS

Foetal heart allogenic grafts were rejected during the first p.o.weeks. Syngenic grafts were able to develop after a early phasis of de-differentiation also characterized by an increase in seric IGF-1. At the end of the first p.o.month cardiomyoblasts seemed to organize into concentric layers. Later on (3-4 months) well formed myocardic layers were to be observed with abundant blood supply confirmed by doppler echography. S-100 staining has shown a general uptake by the implanted and developed cells, so cardiomyoblasts seemed to remain polyvalent regarding to both contractility and conductivity properties. This may explain that the ECG from the implants could be registrated at different delays . It is to be noted that adrenalin and vagal stimulation were able to influence the implant rhythm. 11 months and more p.o. adipose degeneration was observed in the implants. At this moment IGF-1 seric levels did no more significantly differ from the control values (intact animals of the same age and weight).

CONCLUSION

1. Foetal heart syngenic implantation may give growth to partially differentiated and potentially functional myocardial tissue.
2. Further experiment are required to prove whether foetal heart implants may be of use in the repair of adult myocardial alterations

The model of foetal heart implantation and ectopic growth may be used for fundamental studies about development and involution of the heart in physiological and pathological conditions.

OP22 - ANALYSIS OF PIGMENTED SKIN LESIONS USING A COMPUTER ASSISTED MORPHOMETRICAL SYSTEM.

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Pigmented lesions are the most common skin lesions. Most frequent among them is the common acquired nevus, especially intradermal nevus. Dysplastic nevi are benign lesions, but many studies have shown that they are also very important precursors of malignant melanoma. Nowadays, this is even more significant since the incidence of melanoma is constantly increasing. For this reason, it is very important to develop new methods to recognise the malignant potential of precursor lesions.

PATIENTS AND METHODS

In this study, 20 intradermal nevi, 20 dysplastic nevi and 20 malignant melanoma were analysed. Three microscopical were recorded (magnification 40x). Circumference and area of nuclei were measured. The number of nuclei was counted in each field (PC software ISSA – Vamstec, Zagreb, Croatia). Analysis of the obtained data was performed using Poisson's distribution and machine learning systems.

RESULTS

Nuclei of malignant melanoma are the biggest and they have the largest nuclear roundness factor. Poisson's distribution showed that nuclei of examined groups have different way of distribution in space. Machine learning systems gave very high level of accuracy of the classification by using measured parameters (app.95%).

CONCLUSION

Our results indicate that morphometrical parameters combined with complex statistical analysis systems can produce a potentially useful diagnostic help in the analysis of pigmented skin lesions.

OP23 - THE PROGNOSTIC RELEVANCE OF DNA PLOIDY ASSESSMENT IN ORAL LEUKOPLAKIAS

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Aims 5-15 % of leukoplakias of the oral epithelium are dysplastic and one-third develop oral squamous cell carcinomas (OSCC). Recent evidence from molecular pathology indicates that aneuploidy is an early event in the process of carcinogenesis. DNA image cytometry was therefore used to find out if DNA ploidy assessment could identify persons with leukoplakia and a high risk to develop OSCC.

METHODS

DNA ploidy assessment was performed after isolating nuclei of 50 um thick sections, Feulgen staining and measuring with a high resolution image cytometry system (Fairfield Imaging Ltd, London) on 150 patients with leukoplakia typed histologically as mild, moderate, and severe dysplasia and an annual follow-up. Aneuploid cases were assumed to be of poor prognosis in contrast to di/tetraploid cases. The survival data (mean observation time of 103 months) were compared to the histological grading and the DNA classification.

RESULTS

36 of the 150 cases developed cancer, 105 were diploid, 20 tetraploid and 25 aneuploid. Of the 105 diploid cases only 3% developed cancer in contrast to 84% of the 25 aneuploid cases. 60% of the 20 cases with tetraploidy also developed cancer. In contrast to ploidy assessment the prognostic value of histological grading was not significant

Our results will be discussed in the light of recent findings showing a molecular basis of the early occurrence of DNA ploidy changes and a correlation to the number of chromosomal aberrations and to centrosome disorders.

CONCLUSIONS

Our findings indicate that among patients with oral leukoplakias high risk individuals are identified with a considerably degree of certainty by DNA ploidy assessment.

Die prognostische Relevanz der DNA Ploidie Bestimmung von Leukoplakien der Mundhöhle

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OP24 - NUCLEAR CHROMATIN TEXTURE ANALYSIS AND ITS USE IN MAPPING PROGRESSION OF PROSTATIC NEOPLASIA

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Classification of benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia (PIN) and cancer (PCa) a poorly reproducible process. Subtle changes in nuclear chromatin arrangement can be detected using computerised image analysis. Previously this has been shown to be sensitive to early neoplastic change and malignant progression. This study was designed to explore nuclear chromatin texture in BPH, PIN and PCa.

In this study, whole mount histological sections were assessed from a total of 50 patients, and classified as exhibiting BPH (n=26), PIN (n=12) or PCa (n=12). Tissue was stained for DNA with Feulgen and digital images recorded using a calibrated videophotometer system. A total of 1213 nuclei were segmented (618 BPH nuclei, 327 PIN and 266 Cancer) from the images and a series of 60 texture features analysed using software developed in our laboratory.

Analysis showed that nuclear chromatin characteristics were most similar between BPH and Cancer increasing in values for PIN. Discriminant analysis of BPH and Cancer nuclei highlighted nine texture features that discriminated between the two groups. Combining these features into a discriminant coefficient gave an 85.1% correct classification of nuclei.

Discriminant coefficients were calculated for PIN nuclei. When a threshold of zero to distinguish between BPH and Cancer was used, 16.8% of the PIN nuclei were classified as BPH and 83.2% as Cancer. This suggests that PIN nuclei are more similar to Cancer cells than BPH. These results were confirmed by calculating an abnormality index based on Euclidean distance from a BPH baseline vector.

Nuclear chromatin texture offers interesting clues regarding the progression of disease in prostatic neoplasia. An interesting observation is that a monotonic trend from BPH to Cancer is not seen but rather PIN nuclei form a distinct morphological group with a highly abnormal chromatin pattern. Further work should determine the molecular basis of these chromatin alterations and their relationship to cancer development.

OP25 - CHROMATIN PATTERN ALTERATIONS QUANTITATED USING IMAGE ANALYSIS ASSOCIATED WITH CELL CYCLE PHASE

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It has been shown previously that changes in chromatin pattern occur in early neoplastic changes and malignant progression. These changes in chromatin arrangement can be detected using computerised texture analysis. This study aimed to clarify if textural characteristics were dependent on cell cycle phase using flow cytometric cell sorting.

Two human prostatic cell lines, PNT1A and PC3, were harvested at 70% confluence, fixed in 70% alcohol and stained with propidium iodide (PI). The cells were analysed on a Coulter Elite flow cytometer and DNA histograms constructed. G_0G_1, S, and G_2M cell populations were sorted, enriched populations checked for purity and cytocentrifuged onto glass slides. Cytospins of sorted and unsorted cells were stained with Haemotoxylin and Eosin. Images were captured using a calibrated videophotometer system and nuclei segmented. A series of 60 texture features were calculated for each nucleus using software developed in the Quantitative Pathology Laboratory.

Visual analysis showed an increase in size of S-phase cells with distinct nucleolar pattern and an increase in granularity in G_2M in both cell lines. This is confirmed by distinct changes in nuclear texture characteristics in each phase, which were statistically significant. The texture feature energy is a measurement of disorder in the nucleus and exhibited a monotonic trend in PNT1A cells, having increased disorder in G_2M. This however, was not evident in the PC3 cell line – S phase cells from this cell line showed the highest degree of disorganised chromatin.

This study has shown that chromatin texture characteristics undergo distinct changes throughout the cell cycle. This may have a role in characterising prostatic tumour cells and cell cycle alterations based on chromatin morphology. This provides potential information about the tumour behaviour for diagnosis, measuring response to therapy and predicting response.

OP26 - SYNTACTIC STRUCTURE ANALYSIS AND STRUCTURAL ENTROPY AND THEIR CLINICAL SIGNIFICANCE

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BACKGROUND

Syntactic structure analysis can be performed on immune histochemically and ligand histochemically stained digitized images if the staining intensity of cells or different circumscribed compartments are defined as basic structural elements, and an appropriate neighborhood condition is applied. Prerequisites are appropriate segmentation procedures and a consistent texture definition to evaluate spatial relationship between the basic structural elements.

THEORY

In biology, it is of advantage to separate structure from function, i.e., spatial and time-dependent relationships of elements which are characterized by limited expansion of their "inner" energy "tranfer modes". In other words, they posses a boundary which separates them from their environment and forms a system in which quasi-stationary states are created with minimum energy efforts for structures needed. In derivative, the energy needed to create a texture is a minimum, if basic elements are of identical features and located in identical distances from each other, i.e., if we have a "regular" texture without local disturbances. The derived basic formula of structural entropy takes into account distance dependent energy forces ($1/mm^2$) and reflects to the definition of entropy in thermodynamics. If the inner and outer boundaries of a texture (for example solid cancer) are known the current of structural entropy (exchange of energy through the surfaces) can be computed.

MATERIAL AND METHODS

To analyze the clinical significance of the concept of structural entropy and its current, the survival rates of patients with a broad spectrum of intrapulmonary malignancies were set into relation of the calculated entities: Sixty patients with primary colon/rectum carcinomas and their corresponding metastases; eighty patients with pulmonary carcinoids; sixty patients with primary non-small cell lung carcinomas; sixty-six patients with small cell lung cancer; thirty-four patients with primary breast carcinomas and their corresponding metastases, and 50 patients with primary carcinomas of the testis and their intrapulmonary metastases.

RESULTS

In each of the cohorts, the survival of the patients and the disease-free interval between surgery of the primary and of lung metastases was significantly correlated with the amount of structural entropy. Of specific importance are the binding capacities of mammalian lectins, especially galectin-1 and galectin-3, and expression of p53.

CONCLUSIONS

The concept of structural entropy which is a measure of local heterogeneity of cancer cells can be used to estimate the prognosis of patients with various types of intrapulmonary malignancies.

OP27 - FROM TELEPATHOLOGY TO TELERADIOLOGY NETWORK IN CROATIA

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Telepathology as a very demanding branch of telemedicine poses real challenge to experts. Introduction of telepathology in underprivileged countries with poor infrastructure and low health-care budget represents a special task. On the other hand these countries would mostly benefit by introducing telemedicine/telepathology. In our experience it is possible to build an efficient telepathology/teleradiology network using simple POTS and still image transmission, as well as store and forward mode of operation. Also it is important that the applied system has a maximal scalability and support future introduction of faster infrastructure (such as ISDN or ATM). Our experience is based on 7 years of telepathology in Croatia leading to a national teleradiology network. The system used in this development was the ISSA/PHAROS system (Vamstec, Zagreb) integrating patient database with telepathology system. In this paper the ideas, development and software solutions in the process of establishing a national telepathology and teleradiology network are highlighted.

OP28 - MEDICAL DISTANCE LEARNING IN PATHOLOGY USING STRUCTURED REPORTING

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In order to optimize accessibility for online documentation and distance learning in the framework of General Pathology Training at the Medical Universities of Oviedo (Spain) and Cluj-Napoca (Romania), a web application named "LearNetPath" was implemented.
The structure of "LearNetPath" aims to be an easy to use tool, organised on two training levels (one for doctors and one for students), using the universal standard of classification in pathology - SNOMED (Systematised Nomenclature of Medicine).
The web application manages the internet access to images and text data of pathology cases stored in two databases, one for cases written in English and one for cases written in Spanish.
It also provides a module for distance uploading of text and image data of new pathology cases to the server databases.
The clear, quick and easy way of accessing data through internet about a steadily increasing number of Pathology cases in its databases, recommend "LearNetPath" as a powerful tool for training, as well as a tool for international sharing of interesting cases or Pathology studies from across the world.

LearNetPath is available at http://falcon.medicina.uniovi.es/LearNetPath/

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OP29 - PRINCIPLE AND METHODS OF ABSOLUTE EVALUATING INDEXES ON QUANTITATIVE DIAGNOSTIC TEST

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As we know that the predictive values of diagnostic test means the probability that a case suffering a disease when a diagnostic test result is positive or negative. In fact, what we interest is whether an independent case is really suffering a disease or not, but probability. So the aim for the study is to evaluate the absolute values of positive or negative results of diagnostic test to an independent case, not probability. Based on the principle and methods of diagnostic test and on the need for the absolute values of positive and negative results of diagnostic test, the definitions on absolute evaluating index for quantitative diagnostic test were defined first, and then the relative formulae for calculating the indexes were deduced. Results: The diagnostic threshold and region which make all of unsuffering cases negative (false positive is 0) were separately defined as absolute positive diagnostic threshold (APDT) and absolute positive diagnostic region (APDR). The diagnostic threshold and region which make all of suffering cases positive (false negative is 0) were separately defined as absolute negative diagnostic threshold (ANDT) and absolute negative diagnostic region (ANDR). The positive predictive value, negative predictive value, sensitivity and omission diagnostic rate, obtained under the condition of APDT, are separately defined as absolute positive predictive value (APPV), conservative negative predictive value (CNPV), absolute diagnostic sensitivity (ADSe) and absolute omission diagnostic rate (AODR). The negative predictive value, positive predictive value, specificity and mistake diagnostic rate, obtained under the condition of ANDT, are separately defined as absolute negative predictive value (ANPV), conservative positive predictive value (CPPV), absolute diagnostic specificity (ADSp) and absolute mistake diagnostic rate (AMDR). Under the separate conditions of APDT and ANDT, the diagnostic accuracy of diagnostic test is defined as confirmed diagnosis rate (CDR), and the sum of ADSe plus ADSp is defined as absolute diagnostic index (ADI). Based on the above definitions, all of the evaluating index which are acquired under the separate conditions of APDT and ANDP are called absolute evaluating index (AEI) in this paper. Formulae respectively for APPV, ANPV, CPPV, CNPV, ADSe, ADSp, AODR, AMDR, CDR and ADI have been setup, and CPPV, CNPV and CRD have been standardized. The significance of APPV, ANPV, CPPV, CNPV, ADSe, ADSp, AODR, AMDR, CDR and ADI is interpreted. Conclusions: It is useful for APPV, ANPV, CPPV, CNPV, ADSe, ADSp, AODR, AMDR, CRD and ADI to evaluating and diagnostic test and to answer that whether a case suffer or unsuffer a disease.

KEYWORDS

Diagnostic Test, Absolute Evaluating Index, Absolute Positive Diagnostic Threshold, Absolute Negative Diagnostic Threshold

* The Study was supported by National Natural Science Foundation of China

OP30 - ANALYSIS OF THE PARKIN GENE IN PATIENTS WITH EARLY-ONSET (JUVENILE) PARKINSON´S DISEASE

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Mutations in the PARK2 gene (cromosome 6), encoding parkin, have been found in patients with an early-onset familial (recessive) form of Parkinson´s disease (PD; OMIM 602544). Parkin has an ubiquitin-protein ligase activity for several proteins, including alpha-synuclein (mutated in the dominant form of PD). Mutations in the PARK2 gene would abolish this activity, and protein degradation would be impaired. The accumulation of protein aggregates in brains of patients with PARK2-mutations would be neurotoxic for nigral neurons.

We analysed the coding sequence (exons 1 – 12) and 450 bp of the promoter region of PARK2 in 15 patients with and early-onset (<35 years) PD. In the 15 cases, both parents were healthy. Two patients had at least one affected brother/sister, thus being familial forms. In 13 cases, the patient was the only affected in the family. To search for mutations/polymorphisms, genomic DNA from each patient was PCR-amplyfied, subjected to SSCP analysis and, if necessary, sequenced.

We found several PARK2 polymorphisms, previously described. We also identified a new common polymorphism in the promoter region, that would be useful in association studies of diseases in which parkin could be involved.

None of the 13 sporadic cases had mutations. A 2 bp deletion (exon 11) was found in one of the 2 familial forms. This frameshift mutation introduces a stop at codon 394. The family consisted of three affected siblings (onset before 23 years), born to first-degree consanguineous parents. The three patients were homozygotes for the 2 bp deletion.

In conclusion, mutations in the PARK2 gene are found in patients with an early-onset familial form of PD. Mutations in this gene would be rare in patients with an early-PD and without a family history of the disease.

OP31 - QUANTITATION OF ERB-B2 POSITIVITY FOR EVALUATION OF HIGH RISK PATIENTS

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BACKGROUND

We tried to find the most appropriate method for evaluating the erbB2 immunopositivity for detecting the patients who would be at the highest risk of dying of breast cancer.

METHODS

We immunostained 317 breast cancer samples for the erbB2 oncoprotein, and determined the immunostaining index based on the intensity of staining and the fraction of cells stained. The immunostaining index, and tumor size, standardized mitotic index (SMI), and bcl-2 status were also studied and used in univariate analysis. Further, Cox´s multivariate meted was used for finding the features most suitable for prognostication. To find the optimal cutpoints for erbB2 immunostaining index various values of the index were systematically tested. Risk ratios of dying of breast cancer were determined, and the effect of bcl-2 status on survival in the high risk group was studied. More traditional ways of evaluation of the erbB2 positivity were also analysed.

RESULTS

In lymph node positive patients the cutpoint at the erbB2 index 1.5 showed the highest difference in survival. There was a correlation between erbB2 positivity and bcl-2 negativity. In univariate analysis, erbB2 immunostaining showed significant prognostic value in postmenopausal, node positive, and postmenopausal node positive patients, and in the two latter groups, erbB2 was even more powerful prognostic factor than the SMI. After multivariate analysis erbB2 was the most powerful prognosticator among postmenopausal N+ patients. Bcl-2 positivity was associated with survival among erbB2 positive patients.

CONCLUSIONS

The erbB2 immunostaining index gave prognostically more significant evaluation than the traditional interpretation. Strongly erbB2 positive patients had a high mortality. Among the latter group bcl-2 positivity was associated with survival. We suggest that bcl-2 immunostatus should routinely be analysed in clinical erbB2 studies.

OP32 - DOWN REGULATION OF HISTONE H4 ACETYLATION BY NICKEL INHIBITS MDR1 GENE EXPRESSION WITHOUT ALTERATION IN NUCLEAR TEXTURE IN HUMAN OVARIAN CARCINOMA CELLS.

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We showed previously, by image cytometry, that human ovarian carcinoma multidrug-resistant OV1-VCR cancer cells displayed, as compared to the parental IGROV1 cell line, nuclear texture changes compatible with a chromatin decondensation. These changes were associated to an increased (about 5 fold) DNase I sensitivity in OV1-VCR nuclei, suggesting an increased chromatin accessibility. It has been shown that high levels of chromatin acetylation across complete chromatin domains induced chromatin changes detected as “general DNase I sensitivity". By western blotting, the level of acetylated histone H4 appears increased in OV1-VCR cells. Furthermore, treatment of IGROV1 sensitive cells with the histone deacetylase inhibitor trichostatin A (TSA) induces an increase in histone H4 acetylation level, and a significant expression of *mdr1* mRNA. As nickel salts have been described as inhibitors of histone H4 acetylation, IGROV1 and OV1-VCR cells were treated with 1 g/cm^2 of nickel chloride or nickel subsulfide for 24hrs. This treatment strongly inhibits histone H4 acetylation in both cell lines, and significantly down regulates the *mdr1* gene expression in OV1-VCR cells. However, the effects on the nuclear morphology appear strikingly different. TSA treatment induces a partial decondensation of the chromatin supraorganisation, whereas nickel salts do not affect nuclear texture, suggesting different levels of action.

This work was supported by ARERS and the Comité Départemental des Ardennes de la Ligue Française contre le Cancer.

OP33 - CELL CHANGES CHARACTERIZING EARLY STROMAL INVASION IN UTERINE CERVIX CANCER PROGRESSION

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Early stromal invasion (ESI) rappresents a histological diagnostic category usually included in the conventional category of microinvasive carcinoma (MIC). For cervical cancer, it has been first described by STODDARD in 1952, and further delineated by FENNEL in 1953, BAYARDI et al. in 1957, and FIDLER et al. in 1959. Although ESI has distinctive hystologic characters, it is not considered separately in FIGO and SGO staging, but it is included in the diagnostic category of MIC as part of stage 1A1.
To clarify the morphological and biological changes occurring in the cells sorting from HSIL lesions to start ESI, we studied 200 cases of cervical lesions including premalignant lesions and MIC. The structural changes were evaluated quantitatively on 5 m thick sections stained with Feulgen and E&E. In addition, immunocytochemical analysis for p53, p21, p27, bcl-2, p63, p14 and Ck14 has been performed.
The results, shown in table 1, point out that the ESI cell populations differ significantly from those constituting HSIL lesions from wich they arise. The main changes consisted in a) the disappearance of the phenotypical heterogenity of HSIL, having ESI population a striking homogenity; and b) an almost normal content of nuclear DNA opposed to the absolutely abnormal one of HSIL cell populations. Besides, ESI cells have a higher mitotic index compared to that of HSIL. The cells of microcarcinoma (MIC) have a higher mitotic index, toghether with a more abnormal DNA nuclear content and an increasing phenotypical heterogeneity.
The immunohistochemical study further characterizes the ESI cells as cell population very different, from HSIL cells, for the p53 expression associated with p21 and bcl-2 negativity, just the opposite behaviour compared to HSIL.
In conclusion, ESI represents a separate diagnostic category based on objective, measurable data, which has also an objective definition (less than 1 mm invasion in depth). According to BURGHARDT et al. (1997), ESI also has a different prognosis with respect to MIC (stage 1A1: depth of invasion between 1 and 3 mm) and stage 1A2 (depth of infiltration more than 3 mm and less than 5 mm) in wich fatal recurrencies occur in 1.2% and 1.7%, respectively, compared to that of ESI wich equals 0.2%.

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OP34 - QUANTITATIVE ASPECTS OF MYOMETRIAL MODIFICATIONS AFTER BILATERAL LIGATION OF UTERINE ARTERIES

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Most frequently, the loss of arterial blood supply in a tissue results in ischaemic modifications and even necrosis. The uterine system acts as a barrier situated on one of the main branches of internal iliac artery. We aimed at a quantitative study of the histopathological modifications in uterine structures that occur after bilateral ligation of the hypogastric artery, compared to a control group. The recordings performed pointed out that the most typical histopathological aspects are found between 24 and 48 hours after the ligation of the hypogastric arteries, while 30 days after arterial ligation the histological aspect completely resembles with the initial one.
In conclusions, in surgical vascular obstructions occurred in hypogastric arterial system, some preformed or newly formed vascular structures become functional and assure the vascularization in visceral segment situated below the level of arterial ligature. Thus, the bilateral ligation of hypogastric arteries in genital hemorrhages which cannot be controlled by medical treatment doesn't compromise the uterine structure, its revascularization depending on angiogenesis or on the opening of non-functional preformed vessels.

OP35 - QUANTITATIVE ASPECTS OF ANGIOGENESIS IN UTERINE ADENOCARCINOMA

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Formation of new blood vessels intra- and peritumoral is one of the essential events in tumorigenesis and the main condition of tumoral invasion. Microvessel density is considered to be a marker of tumoral aggressivity and also a prognostic factor in the evolution of neoplasia. We aimed at a quantitative study of microvessel density in uterine adenocarcinoma of different stage, using the immunohistochemical staining methods. We used hysterectomy specimens prelevated from patients with uterine adenocarcinoma, compared with specimens prelevated for benign conditions (endometrial hyperplasia). Our study points out the differences in microvessel density in endometrial carcinoma stage I and II FIGO compared to normal and hyperplasic condition. The quantitative analyses computed-assisted confirmed the evolution of angiogenesis with the degree of tumoral aggressivity.

OP36 - PAPILLARY VS. TUBULAR TUMOR: A TOPOLOGICAL ASPECT OF HISTOPATHOLOGY. ITS QUANTITATION APPLIED TO THE INTRADUCTAL PAPILLOMAS OF THE BREAST.

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Among the tumors arising from glandular epithelia we experience a series of pattern from papillary (or villous) to tubular tumors. Purely tubular or purely papillary tumors are extremes. In fact, all intermediate patterns are likely to exist between them. We show that the degree to which a given pattern is papillary or tubular can exactly be described by an index, if the picture is treated from the viewpoint of connectivity in a 2-D, 2-phasic pattern comprizing the interstitial and luminal phases, which are delimited by the basement membrane (BM) of epithelia. An index $\tilde{n}_e$ which we call the index of papillarity or tubularity, was introduced based on mathematical treatments of the curvature of BM, the linear interphasic boundaries. In a non-indented, perfectly tubular pattern, it gives a value of +1.0 while in a purely papillary one, –1.0. When $\tilde{n}_e = 0$, it corresponds to an arabesque pattern where interstitial and luminal phases are mutually embracing. Measurement of curvature can easily be performed by tangent counting technique of DeHoff; it involves "sweeping" of a microscopic test area by parallel translation of a test line which creates tangents with the curves. This method was applied to microscopic pictures of intraductal papillomas of the breast obtained from 40 patients by microdochectomy. The values of $\tilde{n}_e$ proved to disperse over a range from –0.48 to +0.65 with a mean of +0.146, showing that in reality, intraductal "papilloma" is, on average, a tubular tumor. Besides, $\tilde{n}_e$ was shown to significantly correlate with $V_v(i)$, the interstitial volume ratio of tumor, giving a clue to understand the morphogenetic difference between papillary and tubular tumors.

OP37 - THE VALUE OF PROGNOSTIC FACTORS IN BREAST CANCER IS DEPENDENT ON THE METHOD WHICH IS USED FOR ESTIMATING THEM

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Prognostication of cancer is an important part in the selection of treatment for cancer patients. Basically the philosophy is this: patients who have worse prognosis can be and are subjected to more intense treatment than patients who have favourable prognosis. Favourable prognosis means that the probability of the patient to die on the basis of his/her disease is low. The probability of patients dying of their disease is higher among patients with worse prognosis.

It is important that the evaluation of prognosis is as reliable as possible.

In association with breast cancer numerous factors are known to have prognostic value. Many of these features are histological and histological prognostication is based on histological grading. This is to a degree subjective and the power of a grading method to distinguish between patients with good prognosis and bad prognosis may differ in different parts of the scale. So the cutpoint between grade I and grade II may show higher significance than the cutpoint between grades II and III. In clinical practice selection of treatment options can be much helped with the evaluation of the prognostic value of different grading cutpoints.

The same principle can be applied in association with continuous variables.

At certain locations of the scale the cutpoints do not have prognostic power. On other locations they might have. For clinically relevant work it is necessary to select the cutpoint so that the prognostic value is clearest. The treatment options should be applied on the basis of the cutpoints showing biggest prognostic value.

Many prognostic evaluations are based on immunohistochemistry. Subjective estimation in four groups (0, +, ++, +++) may show clinical significance at several cutpoints, but these significances can often be shown to be lower than the significances associated with staining indices which try to evaluate the staining intensity more objectively.

OP38 - GEMISTOCYTIC ASTROCYTOMA – GENETIC EVIDENCE OF GEMISTOCYTES NEOPLASTIC NATURE.

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Gemistocytic astrocytoma (WHO grade II) is a variant of low-grade diffuse astrocytoma characterised by the predominance of gemistocytes with large eosinophilic cytoplasm, and a marked GFAP expression. Gemistocytes have been considered as a sign of poor prognosis as gemistocytic astrocytomas are particularly prone to progress to more malignant neoplasm, i.e. anaplastic astrocytoma (WHO grade III) and glioblastoma (WHO grade IV). The biological basis of this unfavourable outcome is unclear, since gemistocytes themselves have a low proliferative activity and may represent a state of terminal differentiation. Moreover, gemistocytes are observed not only in glial tumours but also in a variety of other central nervous system diseases. These findings raise the question whether gemistocytes are true neoplastic cells or represent reactive glial cells.
To elucidate gemistocyte nature we performed p53 mutation analysis in a pure population of gemistocytes and non-gemistocytic neoplastic cells.
In 6 gemistocytic astrocytomas carrying a p53 mutation, gemistocytes and non-gemistocytic neoplastic cells were separately isolated using a laser assisted microdissection system. Direct DNA sequencing showed in all 6 cases identical p53 mutations in both gemistocytes and non-gemistocytes neoplastic cells. In all 6 cases the wild-type allele was not present, and the further analysed non-tumor tissue showed the absence of p53 mutation, excluding the possibility of germline mutation.
The presence of identical p53 mutations in both gemistocytes and non-gemistocytes neoplastic strongly supports their monoclonal origin and that gemistocytes are truly neoplastic cells.

OP39 - MALIGNANCY GRADING OF ASTROCYTIC NEOPLASMS BY QUANTITATIVE EVALUATION OF PAX 5 AND P53 NUCLEAR EXPRESSION

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The WHO classification system of astrocytic tumors indicates the malignancy progression with a conventional grading (from grade 1 to 4) (1) derived from histological and cytological features. Being p53 expression related to cell cycle control and Pax 5 to cell proliferation and tissue development (3,4), the task of our study concerns the evolution of the ability of these biomarkers in detecting the actual grade of malignancy in the main histological categories of astrocityc tumors: astrocytomas, anaplastic astrocytomas and gliobastomas.
For these purpose from the files of the Institute of Pathology of Udine University we selected 46 cases at different diagnosis: 18 astrocytomas, 8 anaplastic astrocytomas and 20 glioblastomas multiforme.
The monoclonal antibody against recombinant Pax 5 has been produced by ourselves (2); for p53 expression we chosed the monoclonal antibody produced by Ilem.
The results are summarized in table 1: Pax 5 expression increases according to morphological malignancy ranging from 0% to 20% (mean value: 7.6%) in astrocytomas, from 1.3% to 6.6% (mean value: 3.87%) in anaplastic astrocytomas, and from 10% to 61% (mean value: 28.18%) in glioblastoma multiforme (5). The p53 expression ranges from 0% to 11% (mean value: 2.96%) in astrocytomas and from 1.4% to 30.1% (mean value: 8.87%) in glioblastoma multiforme.
From our quantitative data it appears that Pax 5 could be considered as a new biomarker useful in evaluating malignancy grade for astrocityc neoplasias.

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OP40 - QUANTITATIVE HISTOLOGICAL ASPECTS OF THE LATERAL PTERYGOID MUSCLE DURING ONTOGENESIS

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The lateral pterygoid muscle develop in the 8-weeks embryo from the mesenchyme of the first pharyngeal arch, in connection with other components of temporomandibular joint, as temporalis muscle, masseter muscle, primitive articular disc. Its evolution was studied on histological samples prelevated from embryos of different gestational age. The histological aspects were analyzed as moment of appearance, localization, structure, evolution and a comparison between the male and female embryos was performed. We conclude that the role of lateral pterygoid muscle and pterygo-discal complex in the function of temporomandibular joint and its disturbances are closely related to their interdependent ontogenetic evolution and there are temporal sex-related differences in their development.

POSTER PRESENTATIONS

PP1 - CORRELATION BETWEEN CHROMOSOMAL INSTABILITY AND CHROMATIN PATTERN ABNORMALITIES IN COLORECTAL ADENOMAS AND CARCINOMAS

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BACKGROUND

Colorectal cancer, like all cancers, is caused by a disruption of vital biological processes due to genetic alterations. Changes in chromatin patterns, detectable by DNA texture analysis, are believed to be among the earliest detectable morphologic alterations which reflect these genetic changes. In colorectal cancer, a major part of these genetic alterations occurs at the chromosomal level, either as numerical changes or as structural rearrangements. It therefore seems plausible that the presence of chromosomal aberrations is related to changes in chromatin patterns.

AIM

To test the hypothesis that changes in chromatin patterns measured by DNA texture analysis are correlated to the chromosomal aberrations in colorectal adenomas and carcinomas as detected by comparative genomic hybridization (CGH).

MATERIAL AND METHODS

In a series of 8 adenomas and 21 carcinomas, DNA texture analysis and CGH were performed. From these cases, a total of 574 adenoma nuclei and 1,741 carcinoma nuclei were analysed for nuclear texture.

RESULTS

Two adenomas (25%) and 10 carcinomas (48%) showed a high level of chromosomal instability (HL-CIN), while 6 adenomas (75%) and 11 carcinomas (52%) showed a low level of chromosomal instability (LL-CIN). In the adenoma group, many DNA texture features showed significant differences between HL-CIN and LL-CIN, including optical density parameters (e.g. MAXOD p=0.000, SKEWOD p=0.000), area parameters (AMHIGH p=0.01), run length features (e.g. RLNU0 p=0.000) as well as several other parameters (e.g. ENTROPY p=0.000, CONTRAST p=0.000, HOMOGEN p=0.000). In the carcinoma group, only 3 variables differed between the LL-CIN and HL-CIN group (MAXOD p=0.000, DENLS p=0.000, and DENDS p=0.000).

CONCLUSIONS

In this small series of tumours, chromosomal aberrations in colorectal adenomas, as detected by CGH, are clearly related to abnormal chromatin patterns as detected by DNA texture analysis. In colorectal carcinomas, this relationship was virtually absent except for a small features relating to the density of the stained nucleus. Colorectal carcinomas that show only chromosomal abnormalities, must have gained the multiple genetic changes necessary for becoming a cancer in another way, which is also associated with changes in chromatin patterns.

PP2 - BARRETT ADENOCARCINOMAS RESEMBLE ADENOCARCINOMAS OF THE GASTRIC CARDIA IN TERMS OF CHROMOSOMAL COPY NUMBER CHANGES, BUT RELATE TO SQUAMOUS CELL CARCINOMAS OF THE DISTAL ESOPHAGUS WITH RESPECT TO THE PRESENCE OF HIGH LEVEL AMPLIFICATIONS

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At the gastro-esophageal junction three different types of cancers occur frequently: (1) squamous cell carcinomas (eSCC) of the distal esophagus, and (2) Barrett carcinomas (BC), and (3) adenocarcinomas of the gastric cardia (CC). The aim of the present study is to investigate in one study whether this peculiar histogenesis is reflected by chromosomal aberrations and related gene expression.
In a single series of experiments 14 squamous cell carcinomas, 24 Barrett carcinomas and 16 cardia carcinomas were analysed.
CGH analysis revealed chromosomal abnormalities in all cases. Typical chromosomal aberrations for the squamous cell carcinoma type were gains of 3q, 11q13, and losses of 3p, 4q, 9p, 11q, 13q. In contrast, typical copy number changes for adenocarcinomas were gains at 2q, 7p, 13q, and losses of 17p. A limited number of other chromosomal aberrations did show significant differences between BC and CC, such as 1q+, but most of them occurred at low frequency. High-level amplifications occurred in all three groups, but the frequency in the CCs was lower than in the other groups.
In conclusion, a general picture emerged in which squamous cell carcinoma frequently showed gains at 3q, 5p, 8q, 11q13, 17q, losses at 3p, 4p, 5q, 11qter, 13q, 18q, and high-level gains at 11q13, 3q24-qter, while adenocarcinomas frequently showed gains at 1q, 8q, 13q, 20, and losses at 1p, 8p, 9p, 17p. In the case of Barrett cancer, this general rule seems to dominate over the squamous origin of the epithelium.

PP3 - ELABORATING NEW SOFTWARE APPROACHES FOR CYTOLOGIC IMAGE ANALYSIS

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Aim for the project it to develop and expand special purpose algorithms performing custom cytological imprint slide image analysis with a focus of dynamic further resulting data evaluation.
These algorithms function as a parallel mechanism for standard image analysis approaches but giving extra view on the problem and extracting additional data more closely fitting current analysis tasks. We bring these algorithms into effect through custom-made software program operating on desktop personal computer. The program tends to use most stand approaches for image acquisition through light microscope with mounted digital colour video camera, it uses standard binary image formats for image data input/output and standard data storage systems. This gives an advantage of placing the program as an interposition module into the complete analysis schema of cytologic imprint slides.
The software detects cell nucleus as an object within an imprint slide view area and determines nucleus morphologic parameters like area, diameter, perimeter, chromatin granularity along with staining intensity of each detected cell nucleus.
An instance of illustration is the data of cell morphologic parameters (including geometrical nucleus/nucleoli parameters and staining intensity) used for further estimation and comparison of breast cancer cell population nuclear grade (NG). This case (with 100 cancer cells for each of 20 ductal carcinomas, stained according to Leischmann-AzureII-eosine method) showed the higher NG (correlation coefficient $r=0.39$, $p<0.001$) with increasing nucleolar area, nuclear roundness factor, nuclear area and chromatin area within the cell nucleus.

PP4 - EGF-R AND EGF COEXPRESSION IN LUNG CANCER.

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Epidermal growth factor receptor is a transmembrane glycoprotein expressed in normal and neoplastic cell of ectodermic origin and it is able to stimulate cell division when is linked to its ligands.

Pepsin pre-treated slides from 105 archival lung cancer biopsies typed after oms-98 histological typing of lung cancer were immunostained with iorr3 (anti egf-r) and egf1 (anti egf) mabs using an abc-complex peroxidase method to evaluate the over-expression of both mabs in lung cancer as well as its correlation with general and histopathological features of patients.

Iorr3 and egf1 mab were intense and non homogeneous immunoreactives. Membrane limited for iorr3 mab but cytoplasmatic for the other one in more than 50% of neoplastic cells. Coexpression of both mabs occurred in 56,7% of tumors mainly in 14/26 squamous cell carcinoma, 1/1 mucoepidermoid and 6/12 large cell carcinoma. In sclc non intense coexpression was demonstrated in 5/10 and 2/12 samples of carcinoid and small cell tumor variant respectively. Coexpression was also predominant in well differentiated carcinoma disminushing with the differentiation degree ($p<0.05$) and recidivant carcinomas (53%).
The overall survival was of 71.4% at 3 years with a median survival time of 34 months. Kaplan-meir curves and log rang test shopvwed that pathological stage ($p=0.0008$), histopathological type ($p=0.0103$), and recurrence ($p=0.001$) represented important factors for survival in univariate analysis but egf-r and its coexpression with egf fail to be prognostic ($p=0.051$). However, they may be taken into consideration as target for both passive and active immunotherapy.

PP5 - A QUANTITATIVE ANALYSIS OF THE GLYCOGEN CONTENT AND ITS FRACTION IN HUMAN HEPATOCYTES AND ITS ROLE IN DETERMINATION OF THE LIVER DAMAGE DEGREE IN CHRONIC HEPATITIS AND CIRRHOSIS

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In previous studies we developed cytofluorimetric method for measuring total glycogen content and its fractions, labile and stabile, in individual cells of the liver parenchyma. This method allowed to use the glycogen content as indication of glycogen-forming function of human hepatocytes (the material obtained by liver punction biopsies) in normality and pathology. We have shown that total glycogen content significant increases in hepatocytes of patients with subclinical forms of chronic hepatitis and cirrhosis parallel with severity of the diseases. Some forms of the liver cirrhosis characterized by a 4-fold increase in the total glycogen content in hepatocytes as compared with normal cells. As well as in chronic hepatitis cirrhosis, content of the stable fraction enhanced to 30-40%, whereas in normally it amounted to 10-15%. Thus, the cytofluorimetric quantitative analysis of measuring the glycogen content in hepatocytes of the patients with liver pathology can be used for diagnostics of subclinical, symptomless forms of chronic hepatitis or cirrhosis.

PP6 - ANGIOGENESIS IN CIN

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Angiogenesis is a known and necessary process required for the tumor growth.

AIM

To determine whether angiogenesis precedes the tumor invasion, and if it can be of any practical use in differentiating cervical intraepithelial neoplasias (CIN) that will invade from those that will remain in situ.

MATERIAL AND METHODS

Fifteen biopsy specimens of the uterine cervix were immunostained for CD 31 and F-VIII and analysed. The number and diameter of blood vessels was determined under the normal and metaplastic squamous epithelium, cylindrical endocervical epithelium and squamous epithelium showing changes from CIN I to CIN III. The depth of blood vessels position with regard to the tissue underlying the basement membrane in each specimen was also noted. The software used was PC software ISSA-2 by «VAMS», Zagreb.

RESULTS

Their summary is shown in the table: Microvessel counts along the basement membrane

	CD 31	F-VIII
SC. EPIT.	**5 (2-7)**	**5 2-8)**
MET.SC. EPIT.	**6 (2-10)**	**6 (2-9)**
CIL. EPIT.	**3 (1-5)**	**3 (1-5)**
CIN I	**12 (5-18)**	**11 (6-17)**
CIN II	**15 (6-23)**	**14 (7-25)**
CIN III	**25 (9-54)**	**24 (9-57)**

No difference in the number and diameter of the blood vessels was observed in the tissue underlying the basement membrane more deeply than 0.1 mm. The striking difference on the 1 mm of tissue length was noted at the basement membrane.

CONCLUSION

The results demonstrate that a region of neovascularization develops along the basement membrane subtending dysplastic epithelium when compared to adjacent normal epithelium. Comparison of microvessel counts underlying normal epithelium, low grade lesions (condyloma and CIN I) with microvessel counts of CIN III lesions shows a statistically significant increase in the more advanced lesions.

PP7 - ROUNDNESS IN NEUROENDOCRINE TUMORS OF THE LUNG

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The most recent WHO classification of pulmonary neuroendocrine tumors recognizes four entities: typical carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma and small cell carcinoma.

AIM

The aim of this study was to estimate nuclear size and roundness in carcinoid tumors and small cell carcinomas of the lung.

METHODS

At Institute of Pathology, University of Nis five cases of typical carcinoid tumor and ten cases of small cell carcinoma of the lung were analyzed on biopsy samples obtained by fiberoptic bronchoscopy. After formaline fixation and paraffin embedding, serial histologic sections were routinely stained with H&E. The nuclear size et roundness were estimated using image analyzer LUCIA M 3.51 ab (Nikon) at objective 40x, after binary image editing. In each case a hundred nuclei were measured. A statistical analysis was performed using Mann-Whitney test.

RESULTS

The roundness of nuclei in typical carcinoid tumor (0.963±0.007) was significantly larger than in small cell carcinoma of the lung (0.908±0.021), $p<0.01$. No significant differences in nucler size were found.

CONCLUSIONS

The authors conclude that nuclear shape is more rounded in neuroendocrine lung tumors of low-grade -typical carcinoids in comparison to high-grade tumors - small cell carcinomas. Further studies on a larger number of patients are required to confirm these findings.

KEYWORDS

roundness, carcinoid, small cell carcinoma.

PP8 - INTRATUMORAL HETEROGENEITY OF AGNOR STAINING IN LARYNGEAL SCC

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Relatively few studies have investigated the expression of AgNOR in laryngeal SCC. Among these studies there are conflicting results concerning the potential prognostic utility of AgNOR parameters. Intratumoral heterogeneity in laryngeal SCC has previously been demonstrated for DNA content. As this is a potential source of highly variable results we decided to investigate AgNORs in different intratumoral locations in a population of SCC of the larynx.

PATIENTS AND METHODS

33 total laryngectomy specimen have been investigated. After standard silver impregnation the slides were analyzed a PC based image analyzer system (SFORM, VAMSTEC Zagreb, Croatia). Two areas were analyzed: invasive tumor margin and tumor center. Assessed were the following parameters: mean AgNOR area (**A**), mean number of AgNOR/nucleus (**B**) and number of AgNOR/100 nuclei (**C**). 100 nuclei were analyzed per tumor per area.

RESULTS AND CONCLUSION

Significant difference was obtained between the values in tumor center and invasive tumor margin for all the investigated parameters (A – p=0.002; B- p=0.007; C – p= 0.008). Our results strongly underline the fact that intratumoral heterogeneity is not to be neglected in studies and in diagnostic approach. Also it underlines the need for studies with consistent material sampling and analysis regarding the macroscopical as well as microscopical areas. In this we suggest that the problem of potential prognostic utility AgNOR and DNA parameters should be accessed in a large scale study using standardized approach.

PP9 - MELATONIN INFLUENCE UPON OVARIAN DURING AGING. QUANTITATIVE ESTIMATION.

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The reproductive axis correct function is neuroendocrine mechanisms dependent.The action of the main pineal gland hormone, the melatonin, upon the neuroendocrine reproductive axis as been described by Puig-Domingo et al.,1992.However the neuroendocrine mechanisms throught which melatonin influences the reproductive axis are not well understood (Blomsquist and Holt, 1992).

In this study we have investigated the possible effect of exogenous melatonin upon ovarian during aging. Female rats in three different reproductive stages were used, young (5 month old) cyclic rats, middle-aged (15 month old) and acyclic (24 month old) rats. These were divided in control and melatonin (150 µgrs/100 grs.BW) treated, for two months.

Inmunohistochemical analysis of p53 and Ki 67 expression were performed by streptavidin peroxidase complex using DAB as chromogen acording to the instructions DAKO, LSAB-2 Kit Peroxidase for use on rat specimens, upon ovarian rats fixed in formol and embedded in paraffin, 10µm sections were cut from the blocks of tissues and mounted on slides.This latter was evaluated the percentage of positively stained ovarian area, using an semiautomatic image analysis (Russ,1991;Martinez-Nistal and Sampedro 1995).

The statistic study was performed by Student-Newman-Keuls test with previous ANOVA analysis was carried out, using the Biostat program.Were considered significant values of $p \leq .05$ (Carrasco, 1986).

The percentage p53 protein intensity of staining demostrated was < 10% and for Ki 67 was not found pathology positive cells.

PP10 - MORPHOMETRIC PROCEDURES: AREA COMPUTATION OF AgNOR AS A PROGNOSTIC MARKER IN TUMORAL PATHOLOGY

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A method for area measurement of nucleolar organizer regions characterized by variable coloring intensity was developed and implemented.

These intranuclear structures were stained by an argyrophilic technique and seems to be valuable markers in determining prognosis of malignant tumors.

Our method uses some clustering techniques for color based segmentation of the desired region. First, the digital image of the probe is preprocessed using a mean filter to eliminate the noise due to the acquisition process. Then a color space quantization based on the SCT/center algorithm is applied. The advantage of the Spherical Coordinate Transform (SCT) is that it decouples the color information from the variable brightness information. Then the color segmentation process is fine tuned using a cubic clustering of the RGB color space. At the end, morphological filtering is applied on the segmented tissue to refine its shape and its area is calculated."

PP11 - DISTROPHINOPATIES: PROPOSAL OF A SEMIQUANTITATIVE METHOD FOR REPORTING IMMUNOSTAINING DEFICIENCIES.

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BACKGROUND

Xp21 myopathies, now collectivelly named dystrophinopaties, show a continous spectrum of severity. Reduction ofdystrophin expression and other associated proteins, have been described. We worked out a semiquantitative method based on HISTOSCORE targeted in the percentage of muscle fibres and their level of immunostaining, instead of reporting qualitative features like diffuse/patchy, continous/discontinous, strong/faint, etc. that are unsatisfactory and highly subjective.

MATERIAL AND METHOD

300 slides with different abnormalities in protein expresion, were immunostained for B-spectrin, dystrophins (dys1, dys2, dys3), sarcoglycans, B-dystroglycan and laminin (Novocastra, Laboratories Ltd.) A tissue section of normal muscle was put on each histological slide for control.

Point counting method (Stereology): An eyepice graticule showing 25 points overlying the field of view at random, was used. The % of points lying over one type of muscle fibre, is statistically proportional to the area occupied by that fibre. In stereological notation: Area fraction=Pp/Pt, where Pp= unmber of points hitting fibres of interest and Pt= total number of points hitting the section.

Scoring system and immunohistochemical index /IHC Index Scores of intensity: 0= no staining, 1= discontinous immunolabeling, 2= weak continous immunolabeling, compared with the normal control, 3= strong continous immunolabeling like the control section.

Histocore % of positive fibres x intensity score (0 to 3). Normal Histoscore= 300

Immunohistochemical index (IHC Index) IHC Index = 100 x Histocore/ 300. Normal 100

The results were tabulated and the Pearson`s correlation test was applied to compare the degree of agreement between two pathologists working separately.

RESULTS

The degree of agreement achieved by two pathologists was high (Pearson`s corelation test, r= 0.90, in evaluating partial deficiencies in immunolabelling.

CONCLUSIONS

We advocate the use of the IHC Index based on Histocore assesment of immunostaining deficiencies of muscle biopsies, for several reasons: 1-. Histocore quantification based on the stereological point counting method, is an objective, quick, and reproducible method. 2-. Numerical data is more suitable for quantitative description of immunostainig deficiencies, as well as for correlation studies between

the defective proteins. 3-. A quantitative method is also more suitable for statistical analysis. 4-. This method was particularly useful in cases with marked heterogeneity of immunohistochemical expression. 5-. Quantitation may be able to increase our knowledge of dystrophinopaties by yielding important insights into the relative weigth given to the various features used in immunohistochemical evaluation, by means of a more analytical, standarized and reproducible way

PP12 - NATIONAL CENTRES OF PATHOLOGY

Zubritsky A. N

Nowadays in the all highly developed countries the necessary conditions seem ripe for the creation of the National Centres for Pathology (NCP) which could function as the “brain” centres as well as being simultaneously offices. Otherwise, the each National Pathoanatomical Service being a system of measures aimed at improving diagnosis, treatment and research, will sooner or later be drowened in the already available and currently increasing flood of information. The aim of creating the NCP is to unite all national pathologists on the ground of their state residence by means of the best telecommunications service, to establish at the state level common standards and requirements imposed upon anatomic pathology (standarization), as well as to unify the Knowledge on pathology (unification). The process of organization of NCP is proposed in the form of brief outlines and considerations for scrutiny and analysis to be followed by a thorough discussion. The creation of such the NCP at a qualitatively new level, which would have no counterpart in the world`s pathoanatomical practise, will undoubtedly be a powerful breakthrough in any National Pathology.

PP13 - ANOMALIES OF CHROMOSOME 7, 8 AND 17, C-ERBB2 AND TP53 GENES IN NONPAPILLARY UROTHELIAL CELL CARCINOMA OF THE BLADDER

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INTRODUCTION

Less than 20% of urothelial bladder carcinomas (UCC) are nonpapillary lesions. These tumours usually have a bad prognosis, despite the treatment with chemotherapy. In all UCC muscle invasive tumours several genetic alterations were frequent. These include aberrations of chromosomes 7, 8 and 17, TP53 mutations and C-ERBB2 amplification. It is known that dysfunction of TP53 gene are related with BCG resistant urothelial bladder carcinomas, and tumours with amplification of C-ERBB2 gene are potential candidates for Herceptin® treatment. The aim of this study is to evaluate the prevalence of these chromosomic abnormalities in nonpapillary tumours.

METHODS

We performed FISH analysis in 21 nonpapillary UCCs, using centromeric probes for chromosomes 7, 8 and 17 (Oncor®) and unique sequence probes specific for TP53 and c-erbB2 genes (Oncor®). Normal mucosas were used to establish the limits of aneuploidy.

RESULTS

We found that 81% (17 in 21) cases had alterations in all chromosomes and genes studied. The most frequent alteration was the trisomy/polisomy. Twenty three per cent (5 in 21) had amplification of C-ERBB2 gene.

CONCLUSION

These results suggest that chromosomal imbalances may be associated with bladder cancer progression. The rate of C-ERBB2 amplification was similar to what is find in breast cancer and the treatment with Herceptin® may be a reliable approach. Because, in bladder cancer, trisomy of TP53 is not an event usually described, we need to study more deeply the meaning of this result, as well as its function.

PP14 - POSSIBILITY CHROMATIN IMAGE ANALYSIS TO SHOW LYMPHOCYTE PARTICIPATION IN CYTOKINES AND SCAM INTERESTING IN INFLAMMATORY NATURE OF ATHEROSCLEROSIS

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In last studies we hypothesize, that cell-cell communication disturbances are an important pathogenetic mechanism of atherosclerosis progression. Here we perform comparative analysis of cytokines, sCAM secretion within lymphocyte chromatin state as possible evidence of inflammatory reactions in atherosclerosis by system of image analysis. Two kinds of responses have been researched: coagulation and fibrinolysis (incubation of blood clot within 6 hrs at 37°C) and standardised viscosimetric flow by rotational viscometer (shear rate 100 1/sec, 60s at 37° C and incubation within 6 hrs at 37°C). Cytokines IL-1α, IL-1β, IL-6, IL-8, IL-10 («Immunotech», France), endothelin-1 and soluble cell adhesion molecules (sCAM): sP-, sE-selectin, sICAM-1, sVCAM-1 («R&D», UK) have been determined by ELISA. The chromatin of lymphocyte nuclei was studied using Computer TV Morphodensitometry (MDM) System «DiaMorph» (Russia) in the smears dyed especially for DNA. We used the system image analysis for lymphocyte functional activity study. MDM is a method reconstructing morphologic characteristics of biologic objects from digital matrix of optical density using digital image processing and an automatic. Primary advantages of the MDM method are its capacity: A) to combine morphometrical measurements such as area and perimeter with the cytophotometric ones such as optical and integral optical density, and B) to analyze the microanatomy of individual chromatin blocks. Thus the TV MDM system by DiaMorph may provide quantitative information on morphology that can be correlated with genome function.

Correlation between changes of MDM parameters and changes of cytokine and sCAM level in 2 tests in comparison to initial level has been studied. After rheological test the lymphocyte nuclei as a whole has been not changed. But inside of it the decrease of chromatin activity happened. Whereas we observed the deep reorganization of nuclei after the coagulation test.

Obtained results are the evidence of lymphocytes role in pro- and anti-inflammatory cytokines reaction and cytokine-like sCAM activity at atherosclerosis.

We established very strong correlation between functional state of lymphocytes and IL-1β, IL-6 and IL-10 (which are the active participants in pro- and anti-inflammatory program in atherogenesis) concentration of patients with atherosclerosis,.

According to Computer TV Morphodensitometry analysis results Endothelin-1, ICAM-1, VCAM-1, sP- and sE-selectin levels were not related to lymphocyte epigenom activity similar in both tests. Perhaps, the other cell-cell communication mechanisms are involved. By our opinion, soluble cell adhesive molecules have functional activity to lymphocytes, but this needs additional proofs.

PP15 - COMPUTER TV MORPHODENSITOMETRY FOR ESTIMATION OF UNFAVORABLE ECOLOGICAL INFLUENCE

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Health state of Belarus' population after Chernobyl Catastrophe is under permanent attention of medical researchers. Medicine needs of methods which allow to estimate the disorders in human organism, especially children organism, which in the future can lead to diseases.

MATERIALS AND METHOD

Our research objects were 2 groups of children aged 6-15, exposed to different kinds of ecological pressing. 1 - chronic influence of low dose of ionising radiation. Estimated total effective dose was 40-320 mSv. 2- children exposed to chronic technogenical influence. 3- Third group - children living in ecological comfort. Blood lymphocyte and neutrophil interphase chromatin was analysed. By system of image analysis "DiaMorph"(Moscow, Russia) the method of Computer TV Morphodensitometry was applicated, permitting to processe the images of lymphocytes, dued for DNA in smears. Analysis of chromatine structure allows to evaluate morphofunctional state of genom in cell nuclei by densitometrical characteristics.

RESULTS

Classical morphometrical and cytophotometrical parameters had not shown statistically significant differences in examined groups. However, by this method we revealed significant changes of interphase chromatin separate components. It manifested in a heterochromatin compaction and an increase of euchromatin loosen, that was typical for chromatin activation, observed early in gene inductors influence. In children from 1 group with dose 320 mSv these changes were more expressive. Moreover, we separated the lymphocytes on 2 subpopulations: small one and medium one, by nuclei square and optical density. It allowed to reveal more significant changes in nuclei of small lymphocytes.

CONCLUSION

Thus, by method of Computer TV Morphodensitometry we found activation of genom in children, living in unfavourable ecological conditions. It was more expressed in chronical low dose of ionising radiation influence.

PP16 - A NOVEL INTRANUCLEAR LOCALIZATION OF BCL-2 AND BAX PROTEINS IN COLORECTAL CANCER APOPTOSIS.

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The family of bcl-2-related proteins comprises both anti-apoptotic and pro-apoptotic gene products. It is generally believed that bcl-2 and bax proteins are predominantly membrane-associated and localized in cytoplasmic subcellular locations. The functional role of their perinuclear and cytoplasmic localization has been studied extensively over the past decade. The present study further substantiates our novel finding of intranuclear bcl-2 immunostaining patterns in colorectal cancer apoptosis (Elkablawy et al., J. Pathology in press). The subcellular localization of both bcl-2 (anti-apoptotic) and bax (pro-apoptotic) proteins were assessed by immunohistochemistry, confocal laser scanning microscopy and 3-D analysis in five human colorectal cancer cell lines (HT115, LS174, WiDr, SW480 and CACO2). H2O2 was used to induce apoptosis in a time and dose-dependent manner. The immunohistochemical staining revealed that both bcl-2 and bax proteins are not only present in the cytosol, but also within tumour cell nuclei undergoing spontaneous and induced apoptosis, and included apoptotic bodies. This was confirmed by the confocal laser scanning immunoflurecence microscopy, which revealed clear apparent increase in nuclear bcl-2 and bax 8-12 hours after H2O2 induction of apoptosis in human colorectal cancer cell lines (HT115, LS174, WiDr and SW480). The 3-D analysis revealed chromatin associated bcl-2 staining patterns in apoptotic nuclei and apoptotic bodies. In conclusion, the staining range of bcl-2 protein family members should be extended to the nuclear compartment of human tumour cells. Further research is needed to elucidate the significance of these nuclear patterns in the regulation of apoptosis in colorectal cancer.

PP17 - IMMUNOEXPRESSIONS OF P21 AND RB GENE PRODUCTS IN NORMAL, HYPERPLASTIC AND CARCINOMATOUS HUMAN PROSTATES

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INTRODUCTION

The rates of epithelial cell growth and death in normal prostate glands are in equilibrium. The signalling pathways that lead to apoptosis or cell growth are beginning to be defined, and a number of proteins have been identified. A comparative study of the expression of p21 and Rb involved in the control cell cycle, was performed in normal and carcinomatous human prostates by semiquantitative immunochemical study.

MATERIAL AND METHODS

Biopsies from 15 normal (NP) and 25 carcinomatous (PC) human prostates were immuhistochemically processed for the detection of p21 and Rb proteins in paraffin-embedded sections by the peroxidase-antiperoxidase method. A histologic comparative quantification of immunolabelling density also was performed for each antibody with an automatic image analyser (MIP4 version 4.4, Consulting Image Digital, Barcelona, Spain).

RESULTS

In NP, immunoexpressions of p21 was absent but Rb staining was positive in isolated epithelial cell nuclei. In PC immunoreactions to the two proteins studied were found in many epithelial and some stroma cells. Immunoreactions of Rb was more higher in PC samples than NP.

CONCLUSION

The increased expression of Rb in PC specimens reported seems to correspond to phosphorylated Rb, which promotes cell proliferation. In PC specimens, the increased expression of several factors that hinder cell proliferation (such as the p21) is not sufficient to control tumour growth, because the regulation of proliferation-apoptosis is defective in the majority of advanced prostatic carcinomas. It is possible that additional administration of p21 would be effective as an attempt of hindering Rb phosphorylation which induces the entry in G1 phase of the cell cycle. In this way, prospective clinical investigation will be necessary in order to find the efficient agent that restore the proliferation-apoptosis equilibrium.

PP18 - CLINICO-PATHOLOGIC OUTLINE, CYTOGENETICS AND OUTCOME OF SECONDARY MYELODYSPLASTIC SYNDROME (MDS)/ACUTE MYELOID LEUKEMIA (AML). STUDY OF 77 CASES.

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This study extends our experience to 77 MDS/AML-s examined cytogenetically.

PATIENTS AND METHODS

48 females and 29 males with median age 63 years (16-85). A previous MDS was found in 33 patients (42.85%), and 14 of them (18%) never developed an overt leukemia. Trilineage dysplasia was found in 31 cases (40%). The most commonly MDS were RAEB and RAEB-t. The FAB AML subtype were: 16 M_2, 15M_5, 10M_0, 6M_6, 5M_1, 3M_4, 2M_3, 3AUL, 2ABL, 1M_7. The previous history revealed: chemotherapy (CT) and/or radiotherapy (RT) in 60 cases, occupational exposure in 6, irradiation in one, surgery for a previous cancer in 4, 3 immunosupresor therapy, 1 ^{131}I and 2 syncronous second cancer. Cytogenetic investigations were carried out using GTG banding, and results were expressed in acordance with the International System for Human Cytogenetic Nomenclature (ISCN 95). The prognostic significance of various clinical, pathologic, and cytogenetic data for survival was evaluated by a Kaplan-Meier estimate and compared by log-rank test.

RESULTS

Mean values were: leucocyte 12.48 x10^9 /L, hemoglobin 88 g/L, platelet 101.6x10^9/L, MCV 95.2 fl, LDH 1282 U/L, peripheral blood blasts 17.9% and bone marrow blasts 47.2%. The karyotype was normal in 14 cases (18%) and disclosed abnormalities in 63 cases (82%). We found 5 cases with "good prognosis" anomalies, 27 (35%) showed less than 3 abnormalities, and 31 (40%) had complex karyotypes. 5 and/or 7 abnormalities were found in 33 (42.8%), and 24 of them (72.7%) were complex karyotypes. The group that developed AML up to 4 years since exposition includes all "good prognosis" karyotypes. Intensive chemotherapy was attempted in 36 patients, and 6 of them were consolidated by auto or allo-BMT. Fourteen of these (38.8%) obtained a complete remission. All but 5 patients have died. MDS (P= .04), leucocyte < 20 x10^9 /L (P= .04) and normal or "good prognosis" karyotypes have shown significance after univariante analysis for overall survival (OS). Only MDS before to overt leukemia and karyotype were the most important and independent prognostic factors indicating a favorable prognosis after multivariate analyses.

CONCLUSION

This study confirms the relevance of cytogenetics for clinical and treatment outcome of s-MDS/s-AML. New AML-s associated aberrations are still being discovered, using both standard cytogenetic and FISH (SKY).

PP19 - COMPARATIVE GENOMIC HYBRIDIZATION REVEALS NEW CHROMOSOMAL ABERRATIONS IN NASOSINUSAL ADENOCARCINOMAS IN WOOD-WORKERS.

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INTRODUTION

The progression to malignancy in sinusal adenocarcinoma in wood-workers occurs over a period of years. The latency period between carcinogen exposure (wood-dust) and appearance of malignancy may be as long as 25 years. Now, we used CGH with the main purpose to study the chromosomal alterations in 11 sinusal adenocarcinomas.

METHODS

Comparative genomic hybridization allows a comprehensive analysis of multiple DNA gains and losses in entire genomes within a single experiment. Genomic DNA from the tissue to be investigated, such as fresh or paraffin-embedded tumor tissue, and normal reference DNA are differentially labeled and simultaneously hybridized in situ to normal metaphase chromosomes. The DNA to be tested is labeled with biotin and is called test DNA. Genomic DNA derived from cells with a normal karyotype is labeled with digoxigenin and serves as an internal control (control DNA).

RESULTS

Of the 11 cases evaluated by CGH, all were informative and many aberrations were detected. The major recurrent gains were detected at chromosome arms 8q (11 cases), 7q (10 cases), 12q (9 cases), 18p (8 cases), 5p, 6q, 12p, 19p and 20p (6 cases), 1p, 3q, 5q, 17p, 17q, 19q, 20q and 22q (5 cases), 9q, 11p and 14q (4 tumors), 1q and 10q (3 tumors), 9p (2 tumors). The segment most frequently amplified was 18p11.1-q11 (5 cases), following 1q21-q22, 7q11, 7q21-22, 12q13 and 12p12 and 12p13 (2 cases). Frequent losses were observed at chromosome arms 10q (8 cases), 18q (7 cases), 8p (6 cases), 5q and 14q (5 tumors) and followed by 2p, 9p, 15q and 17p (4 tumors), 3p, 11q, 12q, 17q, 21q and 22 (3 tumors), 9q, 12p and 16p, 16q, (2 tumors).

The most frequent whole chromosome gains were detected in 19 and 20 (5 cases) and loss in 4, 5, 6, 14, 17, 18 and 22 (1 case). Whole chromosome arm gain was found in 19p (7 cases), 19q (6 cases), 12p, 18p, 20p, 20q (5 cases), 16p (4 cases), 5p, 7p, and 17q (3 cases). Whole chromosome arm loss was found in 17p (3 cases), 4q, 5q, 9p, 16p (2 cases). In one case simultaneous gain of the one (5p, 8q, 17q) and loss of the other chromosome arm (5q, 8p, 17p), indicative of isochromosome formation.

CONCLUSION

Our results provide a first comprehensive view of the genomic changes associated with nasosinusal adenocarcinomas and reveal several new sites of genomic imbalance, indicating the possible involvement of novel oncogenes and tumor supresor genes in the NSA progression.

PP20 - EPSTEIN BARR VIRUS STUDY IN HODGKIN LYMPHOMA

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AIMS

Epstein Barr Virus (EBV) infection in Hodgkin disease (HD) is variable and lower in western countries (30-50%) compared with developing countries . We studied EBV infection in a serie of 88 cases of HD.

MATERIAL AND METHODS

Eighty eight cases of HD from de files of Hospital Covadonga were studied. All specimens corresponded to lymph nodes from non-treating patients. Cases were classified according to REAL classification. Immunohistochemical study included: CD15, CD30 and CD20. EBV in Reed-Sternberg cells were detected immunohistochemically (LMP-1) and in 42 patients RNA in situ hybridization (EBERs) were also carried out.

RESULTS

Patients median age was 34 years (9-78). Fifty three patients were males and 35 females. Only 3 patients were HIV positive. According to REAL 19 cases (21,6%) were mixed cellularity; 4 (4,5%) lymphocyte depletion; 52 (59,1%) nodular sclerosis; 3 (3,4%) lymphocyte-rich classical HD and 10 (11,4%) nodular lymphocyte predominance. Twenty four cases were positive for LMP-1 (latent membrane protein). In all cases, except one, similar results using in situ hybridization (EBERs) were observed. According to histological subtypes we observed positivity in 7 cases of mixed cellularity; 3 of lymphocyte depletion; 13 of nodular sclerosis and 1 of nodular lymphocyte predominance. Twenty six % of patients younger than 45 years and 44% of patients older than 45 years showed EBV expression. Twenty EVB positive cases were male and 4 female.

CONCLUSIONS

EBV were observed in 27% of cases, a percentage lower than other previous studies. Immunohistochemical and in situ hybridization techniques showed similar results. Positivity was higher in mixed cellularity and lymphocyte depletion subtypes, patients older than 45 years and advanced stage disease.

PP21 - APOPTOSIS IN LOCALLY ADVANCED BREAST CARCINOMA. ITS ASSOCIATION WITH PROLIFERATION, HORMONAL STATUS AND SURVIVAL

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AIMS

To study the apoptosis in locally advanced breast carcinoma and compare it with proliferation, estrogen receptor and survival.

MATERIAL AND METHODS

Apoptosis in Seventy cases of locally advanced breast carcinoma from de files of Hospital Covadonga were studied. TUNEL (Tdt-mediated dUTD-biotin nick end-labeling) technique to visualized apoptotic cells was used (APOTAG-PLUS Oncor®). Proliferation was evaluated using an immunohistochemical method (Ki-67-MIB-1). All patients were treated with mastectomia and adyuvant chemotherapy. Medium follow-up of the patients was 72,1 months.

RESULTS

Fifty cases (71,4%) showed less than 10X10HPF apoptotic cells and in 20 cases (28,5%) apoptotic cells were higher than 10X10 HPF. Apotosis was associated with histologic grade ($p=0.01$): tumors with high apoptotic index (AI) were mainly poorly differenciated. We also observed an association with ER ($p<0.01$): tumors with high apoptotic index were more frequently ER negative. Only 20 % of ER negative tumors had low apoptotic index versus 80% of ER positive cases. An association was found between apoptotic index and labelling proliferation index (LPI) ($p<0.01$). High apoptotic index was observed mor frequently in tumor with high labelling proliferation index. Only 10% of tumors with low LPI had high AI versus 90% of tumor with moderate or high LPI. Finally, high AI was observed mainly in patients with shorter overall survival ($p<0.05$)

CONCLUSIONS

Apoptosis in breast carcinoma was observed mainly in tumors with high proliferation index, ER negative and poorly differenciated tumors and shorter survival.

PP22 - NUCLEAR CHROMATIN PATTERN ANALYSIS AND ITS USE AS A MARKER IN THE PREDICTION OF CHEMICAL RESPONSE TO HORMONAL THERAPY

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Response to hormonal therapy in patients with metastatic prostate cancer is variable. Novel biomarkers may allow a precise prediction of response in patients. Objective information is obtained by DNA image cytometry, reflecting subtle sub-visual chromatin patterns. The aim of this study was to explore the changes in nuclear texture in relation to predicting response to hormonal therapy characterised by alterations in PSA values.

Patients presenting with metastatic cancer were included in the study (n=23). PSA values were recorded at diagnosis. All patients were treated with anti-androgen therapy and had sequential follow-up PSA tests. Plots of PSA levels against time were used to group patients according to their differential sensitivity to hormonal therapy. Defined groups were long term sensitive (n=6), insensitive late with both slow (4) and rapid progression (10), insensitive quickly with both slow (0) and rapid progression (n=2) and no sensitivity (n=1).Tissue sections were cut at 4μm, Feulgen stained and digital images of nuclei captured and processed for nuclear texture using software developed in the Quantitative Biomarkers Group, QUB. Forty one texture features were measured per nucleus.

Analysis revealed distinct differences between the five prognostic groups (Kruskall Wallis test, $p<0.001$). Discriminant analysis between response groups 1 and 2 combined (early escape groups) and group 6 (long term responsive group) identified a total of 11 nuclear texture features most important to the distinction of these two groups. These features were combined to define a discriminant score from which 68% of nuclei were correctly classified. Discriminant function plotted for all response groups showed an increasing score from hormone resistant to hormone responsive groups following a near- sigmoid curve. Texture based scoring therefore may allow a more accurate classification of patients prior to treatment, permitting the administration of individual patient regimens.

PP23 - EXPRESSION OF CYCLIN E AND p27^{KIP1} IN CERVICAL BIOPSIES DIAGNOSED AS CIN/CONDILOMA FROM WOMEN WITH ASCUS AND NEGATIVE PCR HPV

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BACKGROUND

Identification of cervical intraepithelial neoplasia (CIN) and its distinction from benign epithelial alterations is based on either subjectively applied morphologic criteria. Nowadays it is widely accepted that HPV is present in every CIN and HPV infection results in aberrant expression of cyclin E and p27^{KIP1}. The purpose of this study is to investigate cyclin E and p27^{KIP1} as possible markers for CIN and so to help in the differential diagnosis with lesions that mimic CIN or condiloma.

METHODS

We studied three types of archival cervical biopsy specimens: 1) 10 cases from women undergoing evaluation from ASCUS, histologic diagnosis consistent with CIN or condiloma and PCR in exfoliated cervical material and biopsy material without ADN HPV and considered, after reviewing histological sections, lesions that mimic CIN or condiloma. 2) 6 cases from biopsies witout CIN or condiloma (negative controls) 3) 11 cases from condiloma and CIN PCR (+) (positive controls). PCR studies were done, with primers MY09/MY11 with 35 amplification cycles in exfoliated cervical material and 50 amplification cycles on biopsy material biopsy. Sections of the biopsies PCR (-) (tissue) were deparaffinized, rehydrated and inmersed in 10mM sodium citrate 5 min in an autoclave, and they were incubated at room temperature 25 min with primary antibodies, cyclin E (dilution 1:40; Novocastra, Newcastle upon Tyne, UK) and p27^{KIP1} (dilution 1:25; DAKO, Carpinteria CA, USA) and visualized using the LSAB detection system (DAKO, Carpinteria CA, USA). Only nuclear staining was regarded as a positive reaction. The cyclin E index and p27^{KIP1} index were expressed as percentages of positive cells/100 epithelial cells.

RESULTS

The mean of cells that showed intranuclear signals with p27^{KIP1} was for the group 1: 14,70%, group 2: 0,67% and group 3: 1,00% ($p< 0,085$) and with cyclin E antibodies was for the group 1: 13,10%, group 2: 31,33%, and group 3: 20,18% ($p< 0,001$).

CONCLUSIONS

Inmunohistochemical analysis with antibodies anti-cyclin E and anti-p27^{KIP1} is useful to detect CIN or cervical uterine condiloma-like lesions, allowing a correct diagnosis and then a proper subclassification in the following sections: prominent halos associated with glicogenated epithelium, prominent halos associated with glicogenated epithelium plus inflamatory atypia, mature squamous metaplasia, inmature squamous metaplasia, reactive/reparative alterations with incomplete maturation, squamous metaplasia plus reactive changes .

AUTHORS