114 Effects of age and gender on peripheral lymphocyte micronucleus

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Aging in humans appears to be associated with genetic instability. The cytokinesis-blocked micronucleus assay (CBMN) is a comprehensive method for measuring chromosome breakage, DNA misrepair, chromosome loss, non-disjunction, necrosis, apoptosis and cytostasis. Age and gender are the most important demographic variables affecting the micronucleus (MN) index and studies report frequencies in females being greater than those in males by a factor of 1.2 to 1.6 depending on the age group. It has been shown that a higher MN frequency directly corresponds to a decreased efficiency of DNA repair and increased genome instability.
The aim of this study is to investigate an association between age and gender and the synergistic effect of both upon MN in peripheral lymphocytes.
The study was carried out in Portugal in a sample of 85 subjects without any occupational exposition. The evaluation of genotoxic effects was conducted by applying CBMN in peripheral blood lymphocytes. Heparinized whole-blood samples were obtained, with informed consent, from unrelated individuals, men and women, stratified according to their age: 20−30, 31−40 and 41−55 years old. The correlation of MN frequency with age in men and women was further analyzed by linear regression.
This sample was constituted by 54 women and 31 men, with age mean of 32.42 years old. The mean of in women was 0.81 and in men 0.71. According to age, the MN mean were 0.47, 1.14 and 0.86 in 20−30 (n = 36), 31−40 (n = 35) and 41−55 (n = 14) age categories, respectively. The correlation in both genders with age and MN frequency was not statistically significant.
The mean of MN was slightly higher in women than in men but not statistically significant. In general, and in conformization with other studies, women appear to reach a threshold of genome instability faster then men. Results about age showed that the age category that show highest mean of MN was 20−30 comparing with the category with the oldest subjects. That result can be explained by the size of last sample that is approximately half of the others.

115 Thyroid cancer and multiple primary tumours in the Belorussian cancer registry

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Background: Thyroid cancer incidence rates have increased sharply in the Belarus since 1986 when Chernobyl disaster happened. Radiation exposure of I^{131} at a young age is a strong risk factor, but otherwise the etiology is unclear. To explore etiologic clues, we studied the risk of thyroid cancer after an earlier primary cancer, as well as the risk of developing multiple primaries after an earlier thyroid cancer in Belorussian cancer patients from 1990 to 2007.
Methods: The retrospective cohort study used a study cohort consisting of 643 693 cancer cases diagnosed between 1990 and 2007. Cases were identified from records of the Belorussian National Cancer Registry and followed for thyroid cancer development through 2008. Proportion and Standardized Incidence Ratios (SIR) of synchronous (latency between diagnosis's less than a year) and metachronous primary multiple thyroid cancers (PMTC) were investigated. Only double combinations were considered (971 PMTC cases: 181 males and 790 females).
Results: More often synchronous thyroid cancer combines with tumours of lung, skin, pharynx and kidney (in males) and with breast, lung, skin and kidney (in females). Metachronous PMTC with first thyroid cancer was more frequently noted with tumours of lung, kidney and leucosis (in males) and with tumours of breast, skin, corpus uteri, kidney and leucosis (in females). Secondary metachronous PMTC were more often observed after tumours of kidney, larynx, skin and Hodgkin's lymphoma (in males) and breast, corpus uteri and skin (in females). In males highest risk of metachronous PMTC was established for combination with Hodgkin's lymphoma (SIR = 18.1; 95% CI 7.8–35.5 − when Hodgkin's lymphoma developed first and SIR = 8.6; 95% CI 2.3–22.0 − when Hodgkin's lymphoma developed secondary). Similar situation was observed for females (SIR = 5.5; 95% CI 2.7−9.8 − when Hodgkin's lymphoma developed first but no cases of PMTC when Hodgkin's lymphoma developed secondary). Apart Hodgkin's lymphoma significantly high risk of secondary cancer was noted for tumours of kidney, rectum and leucosis in males and of lung, kidney, breast, corpus uteri, colon, skin, melanoma and leucosis in females. Significantly high SIR were found for PMTC after tumours in kidney, larynx, pharynx, colon, after melanoma, leucosis in males and after neoplasms of kidney, pharynx, breast, lung, melanoma, bones and leucosis in females.
Conclusions: High association of thyroid cancer with Hodgkin's lymphoma, leucosis, kidney cancer and bones sarcomas could be an evidence of radiation impact (due to treatment or due to environment). High level of synchronous diagnosis of tumours located near thyroid gland could be caused by more intensive medical attention to that area.

116 Analysis of surgical operations in tumour of proximal part of the femur and pelvis

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Background: Assessment of effectiveness of surgical treatment in locally-invasive tumours of proximal part of the femur and pelvis.
Material and Methods: Surgery performed on 50 patients with locally-invasive malignant tumours of skin, soft tissues and pelvis bones and femur upper third. Men − 39 (78%), women − 11 (22%). In 24 patients malignant tumour localized in proximal part of femur, in 20 − pelvis bones and soft tissues, in 2 − buttocks area and 4 − marked metastasis in ilioinguinal lymphnodes with infiltration to pelvis bones. Patients aged from 17 to 53 years, average − 35. Histologically, in 6 patients giant-cell malignant tumour, 9 − osteogenic sarcoma, 12 − chondrosarcoma, 5 − sarcoma Ewing's, 4 − rhabdomyosarcoma, 2 − polymorphocellular sarcoma, 1 − soft tissues angiosarcoma, 1 − leiomyosarcoma, 1 − cynovial sarcoma, 4 − fibrosarcoma of soft tissues, 4 − metastatic affection of ilioinguinal lymphanodes and 1 − metastatic affection of pelvis bones.
Tumour size affection consisted of 13 to 45 cm. Total indications to perform inter-ilio-abdominal exarticulation was shown in malignant tumour of soft tissue and bones of upper third femur with transition to ilium area and pelvis minor, buttocks area tumours with affection of pelvis bones, pelvis bone tumours with spread to soft tissues and magistral vessels, and metastatic spread to ilioinguinal lymphanodes with involvement magestral vessels, nerves and infiltration of pelvis bones.
Patients were examinated clinically, radiology and ultrasonic examinations, dopplerography, angiography, CT, MRT and morphological examination. Inter-ilio-inguinal exarticulation and pelvis minor tissue lymphdissection till aorta bifurcation were performed from front approach, processing vascular-nerve fascicle in retroperitoneal space. Off 50 patients in 7 was performed additionally sacral bones resection. In all patients conducted neoadjuvant and adjuvant polychemotherapy.
Results: In post operation period in 3 (6%) − cases observed pyoinflamatory complications, which were corrected after use of tranguilizers and analgetics within 1−2 months. All patients followed up to 1−12 years. In observation off 50 patients were revealed in 6 (12%) tumour recurrence, 14 (28%) − remote metastasis and 2 (4%) simultaneously revealed tumour recurrence and remote metastasis. Three and five years overall survival consisted of 44.5% and 35% accordingly.
Conclusions: Inter-ilio-abdominal exarticulation is selected method of treatment in local spread malignant tumours of proximal part of femur and pelvis. This method is improved overall survival and quality of patients' life. Before, those patients were considered symptomatic or palliative chemo-radiologic therapy.

117 Integrating molecular oncology into basic medical education in the age of individualized cancer therapy

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Medical practice in oncology is becoming more interdisciplinary and treatment options are expanding, not least by the advent of molecularly targeted therapies. These new therapeutic modalities are often considerably more expensive and less universal than standard treatments, their effectiveness depending heavily on the particular genetic and epigenetic changes in each individual tumour. These new delopments converge towards the concept of individualized cancer therapy according to which the therapy for each patient is chosen among multiple available options according to the status and wishes of the patient on the one hand and the histopathological parameters and molecular properties of the tumour on the other hand.
From a point of view of a practitioning oncologist, entirely new kinds of skill and knowledge are required and these requirements have to be met by changes in medical education. For future generations of clinical oncologists, the ability of interdisciplinary communication in a case-oriented manner, an understanding of concepts and basic facts in molecular oncology, and the ability to acquire and pass on relevant information, are indispensable. Towards this goal, Charles University Medical Faculty in Pilsen and Heinrich Heine University Medical Faculty in Duesseldorf have started a new collaborative educational initiative in teaching molecular oncology to medical students. The teaching method combines classical lecturing with practical case oriented teaching, in which small groups of students discuss particular clinical cancer cases with both molecular biologists and clinical oncologists, acquiring and finally presenting relevant information. Our experiences from the first year of this programme will be presented.

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Poster Session
Translational Research

118 Up-regulated proteins in the fluid bathing the tumour cell microenvironment as potential serological markers for early detection of cancer of the breast

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Background: Currently, there is an urgent need to identify biomarkers that can facilitate the early diagnosis of breast cancer as the disease is easier to treat and cure if it is detected early at a localized stage. Moreover, clinically useful biomarkers are expected to lead to a significant reduction in mortality. Here we describe a detailed gel-based proteomics analysis of the tumour (TIF) and normal interstitial fluids (NIF) collected from 69 prospective breast cancer patients with the aim of identifying abundant cancer up-regulated proteins that are common to most breast cancers and that may represent potential serological markers for the early detection of breast cancer.
Materials and Methods: TIFs and NIFs were recovered within 30–45 min from the time of surgical excision as previously described [1]. Samples were analyzed by 2D PAGE coupled with MALDI-TOF MS/MS and validation of the markers was performed by immunohistochemistry in an independent set of patients using tissue microarrays.
Results. A systematic computer assisted analysis of the 2D gels of 69 TIF samples and controls revealed a set of 26 breast cancer markers that were up-regulated in 90% or more of all the TIFs. Most of these proteins were also identified in a carefully selected TIF sample using LC-MS/MS. The expression of calreticulin, cellular retinoic acid-binding protein II, chloride intracellular channel protein 1, EF-1-beta, galectin 1, peroxiredoxin 2, platelet-derived endothelial cell growth factor, protein disulfide isomerase and ubiquitin carboxyl-terminal hydrolase 5 was further validated using a tissue microarray containing 70 malignant breast carcinomas of various grades of atypia.
Conclusions: The set of 26 breast cancer markers revealed in this study represent potential serological markers for the early detection of breast cancer as a significant number of these proteins have already been detected in the blood/plasma by others. The next step, which will require marker prioritisation, will have to take into consideration expression by other tissues and tumours, detection in the blood using independent assays, as well as availability of specific antibodies.

Reference(s)

[1] Gromov P, Gromova I, Bunkenborg J, Cabezon T, Moreira JM, Timmermans-Wielenga V, Roepstorff P, Rank F, Celis JE. 2010. Up-regulated proteins in the fluid bathing the tumour cell microenvironment as potential serological markers for early detection of cancer of the breast. Mol Oncol 4, 65–89.

119 The E3-Ubiquitine ligase c-Cbl protects cells against oxydative stress – usefulness as a prognostic marker and a possible therapeutic target

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Background: The c-Cbl ($p120^{cbl}$) E3-Ubiquitine ligase is directed against several tyrosine-kinase growth factor receptors and functions as a down regulator of these activated receptors. This protein is also considered as a molecular poly-adaptor susceptible to specifically link to an increasing number of signalling proteins. The combination of these two functions would allow c-Cbl to participate in the internalization of the targeted receptors and activate down-stream effectors. Several works including ours have showed its involvement in apoptosis and transformation, and we report new related leads about c-Cbl, apoptosis and malignancy.
Material and Methods: p120 c-$cbl^{-/-}$ (KO) or c-$cbl^{+/+}$ (KO) mice, treated or not with flutamide and/or castration, were explored for c-Cbl apoptotic androgen-dependent modulation. The apoptotic signalling pathway was assessed for protein or/and mRNA expression and number of apoptotic cells (TUNEL, DAPI experiments). Primary mouse embryonic fibroblasts (MEFs) from c-Cbl KO or WT mice as well as prostate cell line LNCaP were assessed for expression of c-Cbl upon etoposide or hydrogen peroxyde apoptotic conditions. Interference with c-Cbl mRNA was performed for ROS expression in LNCaP. Human carcinoma and sarcoma from diverse origin, benign prostatic hypertrophia, compared to healthy tissues were assessed for c-Cbl expression through western blots or Tissue Microarrays (TMA) *in situ* studies.
Results: We showed through *in vivo* and *in vitro* experiments that c-Cbl functions as an anti-apoptotic regulator in prostate, MEFs or LNCaP. Our studies of KO versus WT MEFs clearly showed that c-Cbl is highly susceptible essentially to ROS, protecting cells against oxidative stress. We then observed in numerous human tumours a high expression of c-Cbl, often associated to an important oxidative stress (tested by the anti-endonuclease APE1). We confirmed (already reported) c-Cbl over-expression in prostate adenocarcinoma, correlated to cancer gravity. We spray out this observation to other tumours, through the *in situ* staining of numerous TMA spots, showing our observation could be widen to several malignant tissues: ovary, uterus, brain, colon, rectum, lymphoma, striated muscle. Preliminary experiments, supported by the known c-Cbl regulatory role in energy expenditure, strongly suggest that c-Cbl could also participate to ROS generation.
Conclusions: c-Cbl could be used as a malignant marker in cancer prognosis. It could likely protect malignant cell against oxidative stress and be used as a therapeutic target in human cancer (US Patent in progress).

120 NF-kappaB, a crucial regulator of the HIF-1alpha and HIF-2alpha response

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Considering the heterogeneity of metastatic tumours and that the progressive transformation of cells requires a multistep process; logic dictates that cancer is ultimately driven by a combination of several signaling pathways that are interconnected.
NF-κB and HIF are important survival factors that have been implicated in tumourigenesis, cancer progression and metastasis. Both can be activated by similar stimuli such as oncogenes, inflammatory cytokines (e.g. TNF-α or IL-1β), reactive oxygen species (ROS) and hypoxia. In addition, NF-κB and HIF share a number of target genes (e.g. VEGF) and are involved in similar responses such as inflammation, growth and survival. Given the potential for crosstalk, this study explores the mechanistic and functional role of NF-κB in the HIF-mediated responses under hypoxic and normoxic conditions.
Experimental procedures include the use of various cell lines, siRNA studies, overexpression, cytokine treatment, as well as chemical inhibition of targeted proteins. Techniques such as quantitative PCR, western blotting, Chromatin IPs, and prediction software have been utilised to analyse the interactions of these pathways at a molecular level.
We have found that under normoxic conditions, following TNF-α stimulation, NF-κB activates the transcription of the HIF-1α gene, which results in upregulation of the HIF-1α subunit. This correlates with increased HIF-1α activity, recruitment to target genes and upregulation of transcription.
In addition, we have found that HIF-2α is also increased following TNF-α in a NF-κB dependent manner. However, NF-κB does not increase HIF-2α mRNA, but acts as an indirect regulator of HIF-2α protein stabilisation. In this study we show that HIF-2α is stabilized via a mechanism dependent on the presence of HIF-1β (ARNT). Interestingly, our data demonstrate that NF-κB dependent regulation of HIF-1β results invariably in the upregulation of HIF-2α levels as well as HIF-1α following either hypoxia or TNF-α stimulation under normoxic conditions.
Taken together our results indicate that NF-κB maintains a multifacetted regulatory function by controlling more than one HIF pathway component transcriptionally, thereby, as a consequence, exerting significant influence over HIF-α protein levels and activity.
Ultimately, these findings support the notion that key regulators of the inflammatory process (NF-κB and HIF) are likely to have therapeutic implications for many diseases, including cancer as NF-κB and HIF constitute an important mechanistic link between chronic inflammation and solid tumours.