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Characterization of bladder tumoral lineages established in vitro

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Introduction: On the basis of the epidemiological data in Tunisia, we were interested in the establishment of tumoral lineages from biopsies of bladder taken from the patients presenting a vesical tumor. Tumoral lineages in culture allow the characterization at the phenotype and functional level. Our objective is to study the cell lines from the point of view of their susceptibility to immunity effectors and to test them towards drugs used in chemotherapy with the aim of understanding the phenomena of tumoral cells escape to apoptose.

Methods: We have undertaken several approaches for the establishment of tumoral lineages which have been obtained after 12 months. The phenotypic characterization of two tumoral lineages was realized by cytometry and immunohistochemical test using monoclonal antibodies specific for cell surface markers, tumoral antigens and various receptors with domains of death.

Results:We observed a reduction of expression of CMH class molecules on the surface of the tumoral cells. This result was expected since it was already described that the transformation into maligant cells is often associated with a reduction of these CMH class, this reduction being a marker of bad forecast.On the contrary, we observed an increase of the expression of ICAM-1 adhesion molecule that is usually correlated with a fast tumoral progress, because it facilitates angiogenesis. In the second stage, we studied the susceptibility to apoptose of the two lineages, using adapted positive control lines; For that purpose, we studied the receivers with domains of death such as Fas, TRAIL and TNF. Apoptose induction in vitro was realized by using the way of the TNF, of Fas L or the TRAIL at the end to understand the tumoral resistance to lysis. At a last stage, we realized the immunohistochimical characterization of the established lineages and biopsies of cancer of patients' bladder recruited at the hospital by means of antibodies used in the immunofluorescence.

Conclusions: This comparison is interesting because it is not obvious that the phenotype of the lineage is identical to the that of the biopsy: the in vitro culture selects clones the most adapted to the culture conditions.

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Polyamine analogue treatment of neuroblastoma

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Neuroblastoma is a malignant childhood cancer of the sympathetic nervous system. It is one of the most common tumours found in childhood cancers with a mortality of 50%. Children with cancer are given the same treatment as adults – surgery, chemotherapy or radiation. This is not optimal as growing and developing children are more sensitive than adults. Our aim is to find chemotherapeutic agents that have fewer or no side effects. Such possible chemotherapeutic drugs are polyamine analogues. Polyamines are essential for all living cells and they are involved in cell proliferation, cell differentiation and apoptosis. Their levels are tightly regulated and increased polyamine levels are a hallmark of cell proliferation. Polyamine analogues reduce cellular polyamine pools without taking over the function of the natural polyamine. In several types of cancer cells decreased polyamine levels result in growth inhibition followed by apoptosis, while normal, healthy cells will survive undamaged. Some of these compounds have shown exceptional efficacy in animal tumour models. Several polyamine analogues are in clinical trails and they have shown surprisingly low toxicity in humans. There are thoughts of using them in the treatment of childhood tumours. In the present project neuroblastoma cell lines are grown as multicellular spheroids. Multicellular spheroids resemble tumour growth in vivo better than cells cultured in monolayer. Flow cytometric analysis has shown that polyamine analogue treatment results in cell death. We are studying multicellular spheroids of two neuroblastoma cell lines, SH-SY5Y and LA-N-1, composed of 10 000, 20 000, 50 000, 100 000, and 200 000 cells. Presently we are cryo sectioning spheroids that have been labelled with bromodeoxyuridine to investigate cell proliferation after treatment with polyamine analogues. The sections will also be used for investigation of the level of hypoxia with antibodies against hypoxia inducible factor 1 (HIF1).

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Expression of S100 proteins in the progression of melanocytic lesions

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Background: S100 proteins are differentially expressed in tumors of epithelial origin, exemplified by a high expression of S100A7 in ductal carcinoma in situ (DCIS) correlated to poor prognosis. Less is known about their expression in melanocytes and melanocyte-derived tumors, of neuroectodermal origin.

Materials and Methods: We compared the expression of seven S100 proteins, using Western blotting, in a panel of cell lines comprising normal melanocytes, melanoma cells, normal keratinocytes and squamous carcinoma cells under different conditions of culture. We also examined the immunohistochemical expression of S100 A7, S100A9 and S100 A10 in a panel of 47 melanocyte-derived lesions comprising melanocyte nevi and melanomas.

Results: S100A1 was expressed at a low level in two studied cancer cell lines, but was absent in their normal counterparts. No differential expression was observed in S100A4, S100A7, S100A8, S100A9 and S100A11. In contrast, S100A10 was downregulated in three melanoma cell lines compared to normal melanocytes. SAGE informatics of NCI 60 microarray expression data cell lines revealed a significant correlation between the expression of S100A10 and the expression of the proliferation marker Ki67. S100A7 and S100A9 were not expressed in any of the melanocytic lesions but strongly expressed in hyperplasic epithelium covering the lesions. S100A10, on the other hand, was variably expressed in the melanocytic lesions with the highest expression in regions with a strong proliferating or differentiating capacity, especially in regions in or near the epidermis.

Conclusion: Our results suggest that S100A10 may have a role in the regulation of proliferation or maturation of melanocytes and may have a potential value as a biomarker of activity.

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Expression profile of genes coding for DNA repair in human pancreatic cancer

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BACKGROUND: Pancreatic adenocarcinoma is the fifth leading cause of cancer death and has the lowest survival rate for any solid cancer. Unfortunately, only 10–15% of patients present with small, resectable cancers. The aim of our study was to compare genes expression profiles of malignant and benign pancreatic masses samples in order to distinguish differentially expressed genes by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) in endoscopic ultrasound guided fine needle aspiration (EUS-FNA) specimens.

PATIENTS AND METHOD: We analyzed 10 genes associated with DNA repair in 26 EUS-FNA specimens including pseudotumoral chronic pancreatitis (n=8) and pancreatic cancer patients (n=16). The final diagnosis was obtained by EUS-FNA cytology analysis, by surgical pathology or 6 months follow-up. Quantitative RT-PCR was performed to measure the expression of these 10 selected genes in EUS-FNA specimens. Our selected mismatch repair genes were: ABL1, ANKRD17, EXO1, MLH1, MLH3, MSH2, MSH3, MSH4, MSH5, MSH6. In order to asses the RNA quality we analyzed the 18S and 28S ribosomal RNA bands integrity by electrophoresis on a denaturing agarose gel. For every sample 100 ng of total RNA were available.

RESULTS: In pancreatic cancer samples we detected a significantly ($p<0.05$) reduced expression of four DNA repair genes (EXO1, MLH1, MLH3, MSH2) than in chronic pancreatitis specimens.

CONCLUSION: Expression profiling is a useful method to identify potential target genes. Molecular analysis of EUS-guided FNA samples in pancreatic cancer appears as a valuable strategy for improving our knowledge of molecular mechanism of cancer initiation and progression.

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Clinical and pathologic characteristics of Gastrointestinal Stromal Tumors in 11 Egyptian patients/implications for surgical management at Cairo university hospitals

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Background: Gastrointestinal sromal tumors (GISTs) are rare, but have clinical relevance. The majority is gastric with diverse symptoms, and there

is difficulty in predicting their biological behavior This study was designed to review the clinical characteristics of surgically treated gastrointestinal stromal tumors at our institution and evaluate their immuno histochemical and pathologic features and correlate finding with surgical management and prognosis.

Patients and Methods : Patients and disease characteristics were studied in a group of 11 cases (9 gastric, one jejunal and one ileal). In addition, the pathologic features, surgical management, and treatment outcome were evaluated.

Results: A preoperative diagnosis was suspected in eight using endoscopy and endo sonography while CT defined local extra gastric spread in one patient. The median diameter of the tumors was 6.6 cm and no liver metastases were detected in any case. Planned cold surgery was possible in 8 of the 11 cases and excision was successful in all. Three cases were operated upon emergency basis. Histological and immunohisto-pathological evaluation confirmed the preoperative diagnosis in all cases. In half of the c-kit positive tumors the lesions were high grade malignant

Conclusion: GISTs are underdiagnosed in Egypt due to their vague presentations, but should be incorporated in the list of causes of GI bleeding. Surgical removal is feasible in most cases and the prognosis is strictly related to tumor size and number of mitoses.

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Trefoil factor family 2 stimulates cell proliferation via epidermal growth factor receptor

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Cholangiocarcinoma (CCA) is a malignancy of bile duct epithelium. The trefoil factor family (TFF), consisting of TFF1, TFF2 and TFF3, plays an important role in restitution and repair of the epithelium and is rapidly up-regulated in response to mucosal injury. However, TFF peptides are overexpressed in several human solid tumors. Our study in CCA patients demonstrated that TFF2 is rarely expressed in normal bile ducts and non-malignant stage but expressed highly in tumor stage and that TFF2 acts in concert with TFF3 for tumor progression. The present study aimed to investigate the effect of TFF2 peptide (rTFF2) on cell proliferation in human CCA cell line, KMBC, which shows no TFF2 expression and to explore the signaling pathway by which rTFF2 induced proliferation. Cell Proliferation in the presence of rTFF2 or epidermal growth factor (EGF) was performed by determining cell viability using Trypan blue reagent. EGFR tyrosine kinase inhibitor, PD130353 was used to abrogate EGF receptor. The result showed that rTFF2 increased the proliferation of KMBC starting at concentration of 5-500 μg/ml and EGF increased proliferation of KMBC by dose dependence. Both rTFF2 and EGF promoted cell proliferation and this effect was abrogated by EGFR tyrosine kinase inactivation. In conclusion, TFF2 stimulates cell proliferation via epidermal growth factor receptor signaling pathway.

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Dependence of some molecular-biological peculiarities of breast cancer cells on the blood plasma homocysteine level

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Background. It is known, that estimation of features of malignant process and choice of adaptive tactic of treatment of patients with breast cancer takes place on the basis of clinical and morphological characteristics (size of tumor, histological type, degree of malignant, presence of metastases, age of patient etc.). Besides this, possibilities of the use of different molecular-biological markers are widely studied for providing of authenticity of prognosis of features of flowline of malignant process. Now the mechanisms of malignant cell transformation are studied in detail. They attract attention of researchers on the physiological mechanisms of adjusting of altered pathways and metabolic processes in tumour cells. The amino acid Homocysteine is a one of such natural factors, influence of which on development of oncologic pathology is only studied.

Objectives: 1) to describe the molecular profile of malignant cells of patients with breast cancer; 2) to detect the methylation status promoters of genes, associated with drug resistance; 3) to define the level of homocysteine in plasma of blood; 4) to set the associative communications between clinical, laboratory and molecular-biological parameters.

Methods. Clinical investigations of 117 patients with breast cancer, immunohistological, methylation-specific PCR (MSP), statistical methods.

Results. It is shown, that methylation status of mdr1 gene promotor correlates with expression of P-glycoprotein (r=-0,69, P=0,01), GSTp – with expression of glutation-S-transferase (r=-0,76, P=0,001), tp53 – with p53 expression (r=-0,57, P=0,05), CDH1 – with E-cadherin expression (r=-0,63, P=0,02). Methylation status of bcl-2 gene promotor doesn't correlate with bcl-2 expression. The main level of homocyteine in blood plasma was 9,75±3,67 (SD). Homocystein level correlates with the age of patients (r=0,31, P=0,009), expression of metallothioneins (r=0,26, P=0,03), E-cadherin (r=0,27, P=0,03) and methylation status of promotor of CDH1 gene (r=-0,69, P=0,002).

Conclusions. Summarizing everything mentioned above, let's emphasized on the following: 1) expression of P-glycoprotein, glutation-S-transferase, p53 and E-cadherin depends on methylation status of promoters of encodings genes; 2) increased homocysteine level stipulated the expression of metallothioneins and E-cadherin, which have independent prognostic value and characterize sensitivity to some antineoplastic drugs and tumor invasive and metastatical potential.

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Thermal injury associated with the genesis of esophageal epidermal carcinoma

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Background: Esophageal squamous cell carcinoma is one of the most common and lethal cancers. Some areas from South America present a high incidence of this kind of cancer. Many etiological factors are associated with this disease in these areas such as alcohol, tobacco and hot maté consumption, causing a thermal injury in the esophagus. However, there is no study on the effect of hot maté on experimental carcinogenesis.

Materials and Methods: The effect of thermal injury caused by hot water adiministration at 70ºC by gavage three times/week either with or without N-nitrosodiethylamine (NDEA) at 1 or 10 ppm in the drinking water of female Balb/C mice (8 weeks-old(was analysed during nine months. The control group received cold water at room temperature. Each group was composed by 5 animals. The evaluation was done histologically with hematoxylin-eosin and molecular analysis was done using gene array.

Results: The animals that received cold water or only NDEA did not present tissue alterations. The group that received only water at 70ºC presented an initial epithelial necrosis that caused an acute inflammation that became almost undetected after 8 weeks. However, with the animals that were treated with water at 70ºC and NDEA, the initial inflammatory process became chronic and resulted in a hiperplasia-displasia-carcinoma sequence. Gene array expression analysis revealed that NDEA, even at 1 ppm, altered the profile of cytokines induced or repressed by the thermal injury.

Conclusion: Our results suggest that the concomitant ingestion of low doses of NDEA and water at 70ºC leads to a chronic inflammation from the thermal injury caused by hot beverage administration, and this resulted in esophageal tumors.

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Study of kinetic hepatic regeneration after partial hepatectomy by radioisotopic method

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Background: Liver regeneration (LR) after partial hepatectomy (PH) is now defined as an orchestrated response induced by specific external stimuli and involving sequential changes in gene expression, growth factors production, and morphologic structure. Much of the research on mechanisms and kinetic of hepatic growth has been done only in partially hepatectomized animals and in hepatocytes primary cultures. The study of the hepatic extraction fraction (HEF) by radioisotopic methods gives