resistance, suggesting a novel strategy for increasing NGEN sensitivity in Bcl-2 overexpressing human leukemia cells.

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Cancer inhibition by normal differentiated cells

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Background: The stem cell theory of cancer states that tumor development is originated in a mutated stem or progenitor cell. Stem cells are susceptible of inhibition when there is no need to proliferate and have the same regulatory pathways as tumor cells. These facts allow us to hypothesize that tumor as well as stem cells could be inhibited by normal differentiated cells.

Materials and methods: In our study, we used Balb-c nude mice and MCF-7 breast cancer cells. The nude mice were divided in two groups (n=10), the control group (CG) and the test group (TG), whose mice were submitted to an epithelial removal. The animals of both groups were subcutaneously injected with β-estradiol and progesterone, every day, for three weeks, to simulate the pregnancy full differentiation of the mammary gland. Afterwards, 2 million of MCF-7 cells were injected in the mammary gland (CG) and in the cleared mammary fatpad (TG), in the respective group. Five weeks later, the tumors were removed and their volumes evaluated.

Results: The median volume of the tumors in the TG (64,6mm^3) was superior to the median volume in the CG (5,9mm^3) with a statistical significance (p = 0,003), using the Mann-Whitney test.

Conclusions: Our results demonstrate that there is an inhibition of tumor development by normal mammary epithelial cells, when we use the MCF-7 tumor cell line. They also strengthen our previous hypothesis about the existence of an inhibitory stimulus of normal cells in the carcinogenesis process and may elucidate different unexplained mechanisms, namely the protective role of pregnancy in breast cancer or the graft-versus-leukemia effect in haematological malignancies.

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Detection of deleted malignant brain tumors 1 and runt-related transcription factor 3 gene expressions in bladder carcinoma

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Background: Bladder cancer, comprises 3% of cancer among women and 7% of men, is the second most common malignancy of the genitourinary system is the fourth most common cause of death from cancer in men and eighth most common in women. Deleted in Malignant Brain Tumors 1 (DMBT1) gene, located at chromosome 10q25.3-q26.1 is highly expressed in alveolar and macrophage tissues. Some alterations in DMBT1 gene are caused in gliomas. Despite, a loss or reduction of DMBT1 expression in various cancers including gastric, colorectal, brain, lung and esophageal cancers, it has not been reported in bladder cancers. Runt-related transcription factor 3 (RUNX3) is a candidate tumor suppressor gene, a Runt domain transcription factor involved in TGF-β signaling. It is localized on the chromosomal region 1p36. RUNX3 gene expression in bladder carcinogenesis is particularly unknown. We aimed to evaluate DMBT1 and RUNX3 gene expression profiles in bladder cancer and how their expressions could be related to carcinogenesis in the bladder and their correlation with clinicopathological parameters.

Material and Methods: Fifty-six paraffin embedded specimens of transitional cell carcinoma of the urinary bladder were used in the study. Total RNA was extracted from bladder specimens and cDNA was synthesized. The quantification of DMBT1 and RUNX3 mRNAs were succeeded according to the manufacturers' instructions by using Lightcycler instrument.

Results: DMBT1 and RUNX3 gene expressions were identified in 100% of bladder carcinoma samples. No significant association was found in these genes expression levels when compared to sex and age. RUNX3 gene expression was decreased non-significantly in high-grade tumors. When DMBT1 gene expression was compared to tumor grades, a significant decrease was detected between grade I and III (p=0,028). We compared the expression results between patients' sex, age, pathologic degree and grades. We found that DMBT1 gene expression was decreased when grade was increased in this research.

Conclusion: A correlation was found between the DMBT1 gene expression and tumor grades. Expressions of tumor suppressors like DMBT1 and RUNX3 genes could be used as diagnostic markers in early detection and prognosis of the bladder cancer. Furthermore, detailed studies including these genes should be performed in protein levels in a large scale study.

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Expression of calreticulin in breast and cervical cancer in relation to clinical outcome

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Calreticulin is chaperone protein of endoplasmic reticulum, found in the cytotoxic granules of lymphocytes and natural killer cells. It is released with granzymes and perforin upon recognition of target cells. Contrary to its previous defined function in efficient interaction between cytotoxic and target cells, it is shown in recent studies that tumor spread could be influenced by calreticulin, which is overexpressed in some cancer cell lines and in some tumors. It is also shown that calreticulin may induce tumor progression, because at the concentration of 2,2 x 10-7 M it completely blocks perforin-mediated lysis, by stabilizing membranes preventing polyperforin pore formation. The purpose of this study was to investigate whether the expression of calreticuiln in breast and cervical cancer exists and, if so, whether that expression is related to clinical outcome and tumor progression. In this study 33 patients with breast cancer and 25 patients with cervical cancer were included. Patients with breast cancer underwent surgery, while cervical cancer patients were treated by radiotherapy. Clinical outcome was evaluated for two years for breast cancer patients and for one year for cervical cancer patients. Expression of calreticulin was determined prior to clinical treatment, by immunohistochemistry, using rabbit anti-calreticulin polyclonal antibody, according to manufacturer recommendation. Among 22/33 breast cancer patients who had expression of calreticulin, three of them developed distant metastases. On the other side, among 12/25 cervical cancer patients with calreticulin expression, four of them had progressive disease. It has to be noticed that those progressive cancer patients with calreticulin positivity were also the only patients with progressive disease in both observed groups of patients. Namely, all patients with progression of malignant disease expressed tumor positivity for calreticulin. Our findings support the state that calreticulin can regulate lytic and cytotoxic function. These preliminary results indicate the need for further investigation related to the role of calreticulin in malignant behavior.

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Over-expression of PRKAR1A in hepatic progenitor cells during cholangiocarcinogenesis induced by liver fluke (Opisthorchis viverrini)

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Background: PRKAR1A, a regulatory subunit of protein kinase A type I (PKA I) which plays a crucial role in cell proliferation and differentiation was found to be overexpressed in cholangiocarcinoma (CCA). To clarify the role of PRKAR1A in cholangiocarcinogenesis, we have studied the expression of PRKAR1A in Opisthorchis viverrini (Ov) and N-nitrosodimethylamine (NDMA) induced CCA in hamster model. Materials and Methods: Syrian golden hamsters were treated with Ov and NDMA to induce CCA and were sacrificed on weeks 1, 4, 12 and 24. The immunofluorescence technique was used for localizing PRKAR1A, PCNA and glypican-3 in liver tissues.

Results: PRKAR1A positive staining was markedly increased in hyper-proliferating bile duct epithelial cells indicated by a proliferating marker PCNA observed for liver tissues that belonged to hamsters induced from weeks 12 to 24. PRKAR1A were prominently positive at week 24 as tumor developed in tumor cells. Interestingly, the liver progenitor cell marker, glypican-3 was coexpressed in PRKAR1A positive tumor cells.

Conclusions: Our result indicates that PRKAR1A may regulate cellular hyper-proliferation triggered by the liver fluke and plays role in cholangiocarcinogenesis by induced the aberrant proliferation and differentiation of hepatic progenitor cells.

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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