excessive or deficient programmed cell death have been linked to a wide array of pathologic conditions. Materials and Methods: In this study, using a Tissue Microarray (TMA) comprising 136 cases of OSCC, we have analyzed the immuno-expression of proteins that inhibit (Bcl-2, Bcl-x) or promote (Bid, Puma, Bad and cytochrome c) apoptosis. The results were quantitatively analyzed using an automated imaging system (ACIS III) which detects levels of hue, saturation and luminosity, converting this signal into a numerical density measurement that ranges from 0 to 256. These numerical results were divided into terciles and the following scores were attributed – (1) low expression; (2) median expression; and (3) high expression. Analysis of the association between apoptosis' proteins levels and the demographic and clinicopathological characteristics of the patients were performed by the Chi-square test. Disease-free survival and overall survival probabilities were calculated based on the Kaplan-Meier method. Results: High expression of Bad was associated with patients below 60 years (p=0.018) and with alcohol consumption (p=0.014). High expression of Bcl-x was associated with the presence of vascular embolization (p=0.043) and low expression of cytochrome c was associated with moderatelly/poorly differentiated tumors (p=0.025). Overall survival in ten years is statistically better in patients who presented median expression of Bcl-2 than in patients with low or high expression (p=0.022). Conclusions: Our data suggests that the expression of apoptotic molecules might be used as a prognostic indicator in oral squamous cell carcinoma. Supported by FAPESP 98/14335-2 and 07/50608-4.

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Molecular study of the differential TSPAN6, TSPAN15, TSPAN17, TSPAN18 and CD82 tetraspanins expression between fibromatosis and metastatic sarcomas

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Background: Tetraspanins are abundantly expressed transmembrane proteins with at least 33 members in humans. Their structure span four times the citoplasmic membrane and possess the ability to associate each other and with a large number of different transmembrane proteins. Reduced expression of these proteins has been frequently reported in metastatic lesions. Although there are a lot of studies on expression of tetraspanins in solid tumors, theirs expression profile or the role of these proteins in soft tissue tumors (STT) is still unknown. Materials and Methods: The mRNA expression of CD82, TSPAN6, TSPAN15 and TSPAN17 was investigated in samples of STT (including fibromatosis, leiomyosarcoma, liposarcoma, synovial sarcoma, fibrosarcoma, pleomorphic sarcoma, and others). Bioinformatic tools were used to analyze microarray data of 102 STT cases. Results: Quantitative RT-PCR (qRT-PCR) for CD82, TSPAN6, TSPAN15 and TSPAN17 was performed as technical for 54 of the 102 STT. The entire array was classified according to STKE database and we looked for functional modules that might discriminate fibromatosis (benign fibroblastic tumor) and sarcomas (with metastatic potential). Linear correlations of expression levels of pairs of selected genes were evaluated in order to find alterations in fibromatosis and sarcomas samples. These changes could be indicative of involvement of these genes interaction in the mestastasis or malignant potential. Adhesion molecules, including tetraspanins and integrins, seem to be a promising functional module. Changes in linear correlation of genes pairs were observed in TSPAN15 in relation to TSPAN6, that showed a negative correlation in fibromatosis and positive in others sarcomas (p

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Natural and synthesized inhibitors of the MAP-kinase pathway - study of their efficiency in vitro

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The protein called PEBP/RKIP is a natural inhibitor of the MAP-kinase pathway (Raf/MEK/ERK pathway) in cells [1]. It regulates Raf-1 and MEK activity by direct interaction with these two kinases [2]. Considering the implication of MAP-kinase pathway deregulation in numerous types of cancer [3], we have decided to study the interaction between PEBP/RKIP and Raf-1 in order to design and synthesize Raf-1 inhibitors based on new molecular data.

In a first time we have developed several tests to measure in vitro the activity of Raf-1 and the cascade Raf-1/MEK/ERK. Three methods are currently used to test Raf activity. Two of them, namely Phosphocellulose Filter Binding Assay (PFBA) and Scintillation Proximity Assay (SPA) are based on the measurement of the incorporation of radiolabeled phosphate in the substrate. The third method is based on homogenous Time Resolved-Fluorescence Resonance Energy Transfer (TR-FRET) (LANCE ultra technology, Perkin-Elmer).

Each assay techniques will be developed and we will dress a comparison between them. Moreover, the first results concerning the efficiency of some natural and synthesized inhibitors of Raf-1 will be presented.

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Expression of c-kit/SCF tyrosine kinase signaling pathway and apoptotic genes in ionomycin/PMA treated Jurkat cells

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The principal objective of our study was to test the regulation of gene expression of SCF/c-kit tyrosine kinase signaling pathway and the genes involved in apoptosis (Bcl-2, Bax and Bak), in ionomycin/PMA treated Jurkat cells.

By the means of quantitative real-time PCR, we measured the c-kit, SCF, Bcl-2, Bax and Bak expression levels in Jurkat cells RNA, extracted from ionomycin/PMA treated and non-treated cells. The expression data were normalized to the expression levels of four housekeeping genes and cDNA concentration.

Our results show that in the Jurkat cells, in the absence of exogenous SCF (c-kit ligand), ionomycin/PMA treatment down-regulates the expression c-kit receptor, and induces moderate up-regulation of pro-apoptotic (Bax and Bak) and pro-survival gene Bcl-2, thus showing that the down-regulation of the c-kit does not disturb the balance between pro- and anti-apoptotic Bcl-2 family genes.

According to our data, SCF has a very important role in the c-kit signaling pathway-mediated activation and proliferation of Jurkat cells.

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Expression of MDGA2 novel gene is downregulated in human tumors

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Introduction and aim: Human MDGA2 (hMDGA1) (MAM domain containing glycosylphosphatidylinositol anchor-2) is homologous to the hMDGA1 gene, which has been identified and characterized in our laboratory in the last few years. MDGA2 gene has been mapped to 14q21 and is highly conserved among mammals. Expression analysis in normal human tissues revealed that MDGA2 is expressed as a 5 kb mRNA in heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. Furthermore, three different transcripts of 5, 7 and 9 kb were detected in human fetal brain. A 5 kb mRNA was also detected in all the human primary tumors analysed (breast, ovary, uterus, lung, kidney, stomach and colon). The purpose of this study has been to analyse MDGA2 expression level in different tumor types, by Real Time Quantitative PCR. Patients and methods: Sixteen primary colorectal cancer tissues, twentythree endometrial cancer tissues and five primary non-small cell lung carcinomas, were obtained from patients who underwent surgery at San Carlos Hospital in Madrid (Spain). As control samples, a pool of eight-ten normal tissues from colon, endometrium or lung were used. MDGA2 expression was analysed in all these samples by Real Time quantitative PCR, using the TaqMan® Gene Expression System from Applied Biosystems. Results: Results have shown a significant down regulation of MDGA2 gene expression, as compared to normal tissues, in eight of sixteen colorectal tumors analysed (50%); fourthteen of twentythree endometrial carcinomas (61%) and five of five lung carcinomas analysed (100%). A similar downregulation of the MDGA1 homologous gene was also detected when MDGA1 gene expression was analysed in the same tumor tissues. Conclusion: Expression of MDGA2 is downregulated in human colorectal, endometrial and lung tumors.