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4.013

URINARY METABOLITES OF PGI_2 IN PATIENTS WITH OSTEOSARCOMA.

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The patients (pts) affected by osteosarcoma (OS) have a poor prognosis for early metastatic diffusion. Tumor metastasis may be facilited by interations of tumor cells with platelets and experimental data show that metastatic deposits occur after platelet activation. Prostacyclin (PGI_2) is known as a strong agent inhibitor for preventing platelet aggregation. In this study we assayed the urinary metabolites of PGI_2 in 5 pts witt OS compared with 10 healty controls. The 6-Keto-$PGF_{1\alpha}$, 6,15-DiKeto-$PGF_{1\alpha}$, dinor-4-Keto-prostaenoic acid, dinor-4-DiKeto prostanoic acid an dinor-4,13-DiKeto prostandioic acid were isoled after HPLC purification and were assayed by immunoenzimatic analysis. The results of this study show that all urinary metabolites of PGI_2 in pts with OS were decreased compared with healty controls ($p<0,001$). These results indicate that metabolisa of PGI_2 in pts with OS is altered and platelet aggregation "in vivo" may be facilited by this antiaggregatory effects. In conclusion, these data suggest tha decreased of urinary metabolites of PGI_2 in this selected group of pts can assume a rilevance potential to pattern of metastasis.

4.014

TUMOR FORMATION BY SV40-IMMORTALIZED HUMAN MAMMARY EPITHELIAL CELLS

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We have defined 3 different stages of transformation for a human mammary epithelial cell line, HuMI, originally obtained by microinjecting SV40 DNA into normal epithelial cells.
HuMI cells were followed for more than 130 passages and sublines exhibiting distinct differentiated phenotypes were selected. Immunohistochemical staining showed that all passages and sublines were strongly positive for the large T-antigen and keratin 18, but did not express CEA. Anchorage independence assays using soft agar and tumorigenicity assays in nude mice have revealed 3 major phenotypes of the HuMI cells. This cell line grew in an anchorage-dependent manner and remained not tumorigenic until passage 80. Later passages exhibited anchorage-independence with a cloning efficiency of up to 2% and gave rise to a slowly progressing tumor in only 1/20 nude mice. Two sublines, selected either by isolating individual colonies of late passages grown on soft agar (HuMI-A4) or by culturing the tumor cells (HuMI-TU) formed rapidly progressing tumors in 3/4 and 4/4 nude mice, respectively.
Our results suggest that immortalization of epithelial cells with SV40 DNA alone may trigger an event ultimately leading to tumorigenicity. They also indicate that HuMI cells may provide a powerful *in vitro* model system for the study of human mammary cell transformation.
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4.015

HOX gene expression in primary and metastatic small cell lung carcinoma.

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Small cell lung carcinomas (SCLC) are composed of cells best characterized as having an immature neuroendocrine phenotype, which resembles that of an endocrine cell found in sparse numbers in the normal bronchial mucosa of adults. The precise embryonic lineage for this cell has been disputed over the years.

Homeobox containing genes are a network of genes encoding DNA-binding homeodomains highly conserved throughout evolution. They are organized in clusters expressed in the developing embryo with a positional hierarchy. In mice and humans, homeobox genes of the HOX family are organized in four clusters on different chromosomes which presumably evolved by duplication of a primordial gene cluster. The order of genes within each cluster is also highly conserved throughout evolution, suggesting that the physical organization of HOX genes might be essential for their expression. HOX genes are expressed, during embryogenesis, in a tissue specific and often stage related fashion. In the adult life, their expression is mostly connected to the central nervous system (CNS).

We analyzed the expression of the four human HOX loci in primary and metastatic SCLC and in normal lung. Poly (A)+ RNA from normal lung and from SCLC was hybridized by Northern blotting with probes derived from each of the 38 human HOX genes located in 4 loci, HOX-1, HOX-2, HOX-3 and HOX-4 on chromosomes 7, 17,12 and 2 rispectively. Results indicate that primary and metastatic SCLC display differential pattern of HOX gene expression wich correlate to the expression of the HOX genes in the CNS.

4.016

α6/β4 Integrin Expression and Metastatic Phenotype in 3LL Cells.

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Since the growth of malignant cells at the secondary sites could depend on the altered adhesion of the cells to substrata, the expression of the extracellular matrix receptors (integrins) was studied. In particular we examined the expression of α6/β4 integrin on some *in vitro* and *in vivo* metastatic variants we have derived from Lewis lung carcinoma (3LL). Binding studies demonstrate an elevated expression of the α6/β4 integrin in highly metastatic 3LL cells. By using different cDNA probes we obtained from an expression library of highly metastatic cells we confirm these data and demonstrate that: *i*) mRNA corresponding to the described entire coding sequence of β4 subunit is expressed only on highly metastatic cells of 3LL, while it is undetectable in lower metastatic ones; *ii*) additional transcripts for the β4 integrin subunit are also detected. Data while indicating that β4 subunit is expressed only in high metastatic 3LL cells, also demonstrate that mRNA coding for β4 subunit undergo alternative splicing.

In conclusion, we suggest that 3LL cells endowed with higher metastatic potential could acquire higher capacity to invade through the expression on their cell surface of specific receptor for cell adhesion.
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4.017

CORRELATION BETWEEN POSITIVITY OF AgNORs AND TUMOR MALIGNITY IN 6 CASES OF non-HODGKIN'S BONE LIMPHOMA

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We applied the AgNOR tecnique to 6 cases of mono-stage non-H's bone limphoma - 2 were diffuse, small clivate cells lesions, 2 were diffuse with large clivate/non-clivate cells and 2 were immuno-blastic cells lesions. All examined samples presented nuclear "dots" or "blebs", in varying proportions. AgNOR granules count was low in the normal lymphoid cells present in control samples. In immunoblastic elements, mean AgNOR value was 5,945 granules per nucleus, whereas the figure was 1,190 in small clivate cells, 1,350 in large clivate cells, and 2,010 in large non-clivate cells. Analysis of the one-way variant was made on the 5 groups of cells examined; statistically significant differences ($p<0,000E+00$) were demonstrated. Moreover, we observed how AgNOR granules count could be correlated with survival time and tendency to invade the surrounding soft tissues - even more than the histologic features evaluated according to the Working Formulation.

4.018

Inhibition of breast cancer derived cell invasion by Suramin in vitro.

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Suramin (Sur), a polysulphonate napthylurea, has been reported as an anticancer and antimetastatic agent. This drug inhibits the binding of various growth factors to their cell surface receptors. It has been reported (1) that Sur has a remarkable inhibitory activity against melanoma cell invasion through reconstituted basement membrane (chemoinvasion). We have investigated the role of Sur on chemotaxis and chemoinvasion (2) to conditioned medium for MCF-7 ras cells, a human breast cancer cell line derived from MCF-7 after transfection of v-Ha-ras oncogene. These cells are malignant *in vivo* (3) and invasive *in vitro* (4). Cells were pretreated with Sur (200 μg/ml) for 48h and, in some experiments, Sur was added also for 6h during chemotaxis and chemoinvasion assays. We have found that Sur highly inhibits MCF-7 ras cell migration (70% inhibition) and completely abolishes invasion in both experimental conditions. We assume that Sur interferes with the binding of some factors involved in the chemotactic and chemoinvasive phenotype. Our data suggest that Sur could be an important anti-invasive agent for malignant cells of breast cancer origin.
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