Symposium no. 4: Biology of Tumour Invasion and Metastasis

4.061

DIFFERENTIATION AND GROWTH IN NUDE MICE OF HUMAN OSTEOSARCOMA CELLS

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We have studied some biologic features of human osteosarcoma on 2 cell lines (U2OS and Saos-2) and 2 new cell cultures (SARG and IOR/OS9). We evaluated parameters of cell growth *in vitro* and the expression of HLA antigens, adhesion molecules (ICAM-1, LFA-3), intermediate filaments and markers of osteoblastic differentiation (type I and type III collagens, osteonectin, alkaline phosphatase). We also studied the ability of these cells to grow *in vivo* in nude mice. Interestingly, only cultures with a low expression of osteonectin and of alkaline phosphatase (U2OS and IOR/OS9) consistently produced tumors after s.c. injection in nude mice. Our results suggest that the degree of osteogenic differentiation *in vitro* could correlate with tumorigenicity of cultered osteosarcoma cells. Further investigations on clinical material will be needed to evaluate the prognostic value of markers of osteoblastic differentiation, like osteonectin and alkaline phosphatase, and of growth in nude mice.

4.062

MEMBRANE RECEPTORS AND ION CHANNELS IN HUMAN SMALL-CELL LUNG CARCINOMA

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We have studied in a number of SCLC and non-SCLC human cell lines the presence and the properties of acetylcholine nicotinic receptors (AChRs) and voltage-operated calcium channels (VOCCs). Binding experiments were performed with nicotinic ligands and with animal neurotoxins such as α-Bungarotoxin and ω-Conotoxin. The receptors were also analyzed by means of polyclonal antibodies. Northern blots were performed in order to detect transcripts specific for different receptor subunits. We have found that SCLC but no non-SCLC cells express nicotinic receptor subunits (α3 and ß4) previously believed to be present only in neurons. SCLC are typically neurosecretory cells and we have recently found they also express an high affinity amine uptake system.
Both nicotinic receptors and calcium channels could be involved in the control of hormone secretion from SCLC cells.

4.063

EFFECT OF TGF-ß ON THE METASTATIC POTENTIAL OF B16 MELANOMA CELLS.

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A cDNA clone for human TGF-ß2 under the control of a strong promoter was co-transfected with the neomycin resistance gene into highly metastatic B16-F10 murine melanoma cells. Stable transformants were selected and those expressing the foreign gene were identified by Northern blot analysis. As control, clones were similarly isolated after transfection only with the neomycin resistance gene. In either case, the growth and morphology characteristics of cells were comparable to the parental population. Lung colonization assays with different clones showed that the number of pulmonary metastases was significantly higher for clones expressing human TGF-ß compared with controls. The results suggest that TGF-ß2 stimulates the metastatic potential of melanoma cells. (Supported by a grant from AIRC)

4.064

Metastatic consecuences of tumor cell behavior during passage through the liver sinusoids. Smith-Zubiaga I. & Barberá-Guillem E.; Dept. of Cell Biology; University of the Basque Country; Bilbao. SPAIN.

In rodents, liver metastasis starts in acinar zone 1 as a result of tumor cell colonies developed in the second quarter of the sinusoidal path (SP). Using BrdU-immunofluorescent microscopy, we have monitored the behavior of portal vein-injected B16F10 and M5076 cells in the liver of C57BL mice, from the first 3 min after injection (AI) until 5 days later. During the first 2h AI, 80% of tumor cells (TC) are destroyed and the survivors remain in the liver sinusoids (LS) more than 5 days. Most of them are arrested in the first half of SP (SP1/2) – the part of LS covered by high glycosilated and manose-receptor rich endothelial cells – and are released slowly. TC arrested in the 2nd half of SP (SP2/2) are released faster. TC caught in LS are out of S phase until a proliferative reaction starts in the sinusoidal cells at 60h AI. Then (SP1/2) replicating TC generate metastasis and SP2/2 replicating TC are released. In conclusion TC able to survive in LS are out of S phase, and liver metastasis efficiency is related with the number of survivor TC and the site where they are arrested in the LS.

4.065

MODULATION OF COLON CARCINOMA-ASSOCIATED ANTIGEN EXPRESSION WITH INTERFERON-γ AND SODIUM BUTYRATE

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Monoclonal antibody (mab) AM-3 detects a mucin carbohydrate epitope whose expression in colon carcinoma is severalfold higher than in normal colon mucosa. The mechanism of this increase was investigated in vitro by treating the HT-29 cell line-derived clones 16.2, 12.2 and 15.2 with sodium butyrate (NaBu) and IFN-γ. AM-3 epitope and a second epitope (AM-7) present on the same mucin molecule were determined on the cell surface with the mabs AM-3 and AM-7 by ELISA. The amount of AM-3 epitope decreased in the order HT-29>16.2>12.2>15.2 and of AM-7 epitope 15.2>12.2>HT-29>16.2. Treatment of the cells with 1 mM NaBu for 2 days or with 10 U/ml IFN-γ for 3 days increased the expression of each antigen as follows:

	HT-29		16.2.9		12.2		15.2	
	IFN-γ	NaBu	IFN-γ	NaBu	IFN-γ	NaBu	IFN-γ	NaBu
AM-3	0	1.4	0	1,5	0	1,7	0	0
AM-7	1.6	2.4	1.9	0	2.9	0	2.0	0

These data indicate independant regulation of AM-3- and AM-7 epitope. The modulation may affect glycosylation rather than the total mucin biosynthesis.

4.066

ORAL HUMAN POLYVACCINES AS INHIBITORS OF SOLID TUMOR METASTASES IN MICE

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The oral administration of the vaccines Respivax (RV) and Broncho-Vaxom (BV) in combination with surgical removal of the primary tumor caused a significant reduction of the number and volume of lung metastases in C57BL/6 mice, bearing implants of Lewis lung carcinoma in the footpad. Treatment with a combination of cyclophosphamide and BV or RV was found to be more effective than treatment with either alone. In immune function studies BV and RV induced an increase in the number of the alveolar macrophages (AM) and rendered AM tumoricidal for syngeneic metastatic cells in vitro, while splenic natural killer (NK) cell activity did not show apparent augmentation. It was found an increased production of interleukin-1 by mouse peritoneal macrophages, stimulated with RV in vitro and elevation of endogenous interferon in the serum after oral treatment.