

11 P

Frequent Loss of Heterozygosity at 6q in Malignant Salivary Gland Tumors
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Terminal deletions of the long arm of chromosome 6 (6q) and translocations involving the same region are the only cytogenetic event common to all histological subtypes of salivary gland carcinomas, but carcinoma ex-pleomorphic adenoma. In an attempt to characterize at the molecular level the frequency and the extension of 6q deletions in salivary gland carcinomas, we have investigated the loss of heterozygosity (LOH) at 6q in a series of 13 salivary gland carcinomas, using polymorphic DNA markers. Frozen tumor samples and the normal tissue from each patient were studied by Southern blotting and dinucleotide repeats analysis, using 13 polymorphic markers (1 at 6p, 1 centromeric, and 11 at 6q). All cases were informative for at least one locus at 6q. LOH was observed in 5 cases, all of them showing different histology. The highest frequency of LOH was found at locus D6S37 (2/6 cases) at 6q26-27. The patterns observed were compatible with terminal deletions in 3 cases and interstitial deletions in 2. Furthermore, the data suggests the existence of two distinct regions of LOH at 6q in salivary gland carcinomas.

In summary, LOH at 6q was found in 38% of the cases in this series of salivary gland carcinomas. Our results support the assumption that loss of genetic material at 6q is a frequent and probably an important event in salivary gland carcinogenesis.

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13 P

ONTOGENY OF HUMAN NATURAL KILLER (NK) CELLS: CYTOLYTIC MECHANISMS. F. Vaz, G. A-Porada, J.L. Ascensão. Portuguese Institute of Oncology, Lisbon, Portugal and Univ. of Nevada School of Medicine, Reno, NV89520, USA.

NK cells are important effectors in graft rejection and against tumor cells. The development of this lytic arsenal during ontogeny remains unclear. We have shown that NK cells can be obtained from human adult bone marrow stem cells and progenitors cultured with SCF, IL-1 α and IL2 (1,000 U/ml) with up to 96% at 4 weeks of culture; in some experiments IL7 (1,000 U/ml) was used instead of IL2. Expression of NK cell markers and lytic activity against K562 targets increased in parallel in cultures with IL2. NK cells grown in IL7 were, however, devoid of lytic activity. We used this system to detect Granzyme A (GA) (a tryptase common to NK and T lymphocytes) and Hu-Met-1 (a met-ase constitutively expressed in mature human NK cells) expression, early (2 weeks) and at the end of culture (4 weeks). After depletion of CD3+ cells, total RNA was extracted and RT-PCR performed. The sense primers were designed to amplify the sequence that codes for the aminoacid residues of the unprocessed protein. The amplified products were detected in an ethidium bromide gel electrophoresis and their specificity confirmed by Southern blot analysis using a specific probe. As early as two weeks, GA transcripts were detected in IL2-cultured cells (CD56+/3- cells: 15.3% $\pm$ 4.9%; % lysis of K562 targets: 10.4% $\pm$ 8.9% at 2.5:1 E:T ratio). In cultures with IL7 GA mRNA was only amplified later in culture (4 weeks). Hu-Met-1 was not detected at any time of analysis in IL2 or IL7 cultures. We conclude that GA expression occurs early during NK ontogeny but its presence doesn't correlate with lytic function. These effectors are immature since Hu-Met-1 expression was not detected; which suggests that other factors may be necessary for terminal maturation of fully cytotoxic NK cells.

15 O

ISOLATION OF AN ESTROGEN RECEPTOR VARIANT WITH INCREASED ACTIVITY FROM PREMALIGNANT BREAST LESIONS.

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Recent molecular evidence suggests that at least half of the premalignant proliferative lesions of the breast have already suffered genetic alterations, suggesting that many of these lesions are in fact neoplasms with an increased propensity to evolve to malignancy. A growing body of literature also suggests that a high percentage of proliferative hyperplastic breast lesions, in contrast to normal breast epithelium, express high levels of the estrogen receptor (ER). Thus we hypothesize that either inappropriate overexpression of normal ER, or expression of certain variant forms of the ER, may drive abnormal proliferation in breast epithelium, providing a favorable environment for the genetic alterations.

We have recently isolated an ER variant from breast hyperplasias with a point mutation within exon 4, which leads to a change of lysine to arginine at the beginning of the ER hormone binding domain. This variant is expressed in more than 30% of proliferative hyperplasias as assessed by single strand conformation polymorphism (SSCP). The ER variant displays increased agonistic properties in transactivations assays in MDA-MB-231 or HeLa cells. It exhibits significantly higher activity levels as compared to WT ER on a vitellogenin ERE reporter, and displays a 200-fold increase in estrogen sensitivity. Furthermore, when the variant receptor is stably transfected into the ER-positive MCF-7 breast cancer cell line, these cells now proliferate in response to very low levels of estradiol.

12 P

INTERLEUKIN-8 (IL-8), INTERLEUKIN-6 (IL-6), TUMOR NECROSIS FACTOR- α (TNF- α), SOLUBLE TNF RECEPTORS (sTNFR) 75 AND 55 AFTER BCG THERAPY OF UROTHELIAL CANCER (TCC): KINETICS OVER 6 INSTILLATIONS

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 The role of different cytokine mediators in BCG therapy of TCC is not well understood. IL-8 is a potent chemoattractant for polymorphonuclear leukocytes. IL-6 is an acute phase protein. TNF- α plays a central role in the cytokine cascade. Soluble receptors to TNF- α have been reported. After intravesical instillation of 120 mg or after perfusion of the upper urinary tract with 360 mg of BCG Pasteur F we measured the expression and time course of IL-8, IL-6, TNF- α and its receptors TNFR 75 and 55 during BCG therapy of TCC. Cytokines were determined by a solid phase double-ligand ELISA/ELIBA method.

IL-8 titers increased to a peak of 1100ng $\pm$ 1250 ($\pm$ S.D.) to 2965 $\pm$ 1560 4-6 h after instillation with a maximum after the 5th. Over 24 h titers were highest during the first 6 h 10184ng $\pm$ 4682. IL-6 attained a peak 4 h after instillations 1-5 (62ng $\pm$ 85 to 125ng $\pm$ 85) and 2 h after instillation 6 (140ng $\pm$ 57). Thereafter it decreased to a steady level. TNF- α reached peak values 1 h after instillation with a maximum (435ng $\pm$ 473) after the third. sTNFR 75 and 55 increase to a peak after 1-4 h. sTNFR 75 peak values vary from 567ng $\pm$ 315 to 1567ng $\pm$ 864, sTNFR 55 from 490ng $\pm$ 398 to 1222ng $\pm$ 1159. In the serum IL-6 titers rise 12-fold and TNF- α titers 2-fold.

In conclusion, cytokines may play a mediating and modulating role in BCG antitumor activity. sTNFR 75 and 55 may antagonise or regulate TNF- α activity. Due to its properties IL-8 may prove to be an interesting parameter for BCG responsiveness.

14 O

NEW MUTATION IN BRCA 1 GENE DETECTED IN 3 AUSTRIAN HBOC FAMILIES

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BRCA1 maps to chromosome region 17q21.1, and mutations in this gene predispose strongly to breast and ovarian cancer. It has recently been isolated and previously it was shown to explain approximately 45 % of all breast cancer families and over 80 % of all families in which both breast and ovarian cancer occur. Women, who carry mutations in the BRCA1 gene, have a lifetime risk of 85 % to develop breast cancer and 60 % for ovarian cancer. Austrian HBOC families with several breast- and ovarian cancer cases were selected for mutation screening on the basis of positive LOD scores. Mutation screening was performed by protein truncation test (PTT). Positive findings were then subjected to DNA sequence analysis. In three until now unrelated families the same mutation could be detected. This mutation in exon 11, 2795delAAAG, has not yet been reported before. The frequency of this mutation in the Austrian population will be of further interest.