476 Characterization of neurotensin, neurotensin receptor 1 and β-catenin in healthy endometrial and endometrioid adenocarcinoma cells

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Background: Endometrial carcinomas are steroid hormone dependent and are characterized by a variety of genetic alterations that include β-catenin gene mutation. This protein acts as a transcriptional activator of the oncogenic Wnt/APC pathway; a target gene of this pathway is NTS1 [1], a high affinity neurotensin receptor over expressed in several other estrogen-dependent cancers such as lung, colon, pancreas, prostate and breast. Given the importance of these molecules for diagnosis, prognosis and therapies, and that its presence in uterus is unknown, our aim was to carry out an immunohistochemical cellular study to characterize the presence of neurotensin immunoreactivity, NT-ir, and its receptor NTS1 in healthy and tumoural endometrium in relation to the subcellular localization of β-catenin.
Material and Methods: Biopsy samples of healthy and tumoural endometrial tissue were taken from 40 women patients at the Hospital Universitario de Canarias. The samples, formalin fixed and paraffin embedded included healthy tissue in secretory and proliferative phases and endometrioid cancer with different grades of differentiation and infiltration. Antisera against NT, NTS1 and β-catenin were used for an immunoenzymatic method.
Results: NT-ir and NTR1-ir were present in healthy endometrium only in stromal tissue and β-catenin-ir was located in the membrane; in the proliferative phase β-catenin-ir was observed in cytosol. In contrast, the tumoural samples show a differential pattern for the three molecules in the glandular tissue in relation to the histological differentiation. In well-differentiated endometrioid adenocarcinoma, the NT and NTS1 immunoreactivity was distributed in both cytosol and nuclei of some glandular cells. β-catenin-ir was always in the membrane.
In moderate and poorly differentiated adenocarcinomas, NT-ir and NTS1-ir increase in number of cells and intensity of immunoreaction, particularly in the nuclei of glandular cells in contrast to stromal tissue. In addition, β-catenin-ir was observed in cytosol and nuclei. We also show that NT/NTS1/β-catenin-ir was located in the nuclei. In addition, there was also a differential immunoreactivity in proportion to the degree of infiltration.
Conclusions: Our observations suggest that the location of NT/NTS1-ir in the nuclei is an early step in the endometrioid cancer progression and that the NT/NTS1/β-catenin-ir coexpression in nuclei could be useful for more accurate diagnosis.

Reference(s)

[1] Souazé, F. et al. Carcinogenesis 27, 708–716(2006).

477 Copy number alterations of glioma cell lines detected by array-based CGH show preferential loss of genetic material and no high-level EGFR amplification

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Background: Tumour-derived cell lines are used as *in vitro* cancer models. Prevoius reports showed that cell lines preserve genetic aberrations unique to the parent histology from which they were derived, while acquiring additional locus-specific alterations. However it remains unstudied whether genomic alterations in glioma cell lines reflect the most common genetic alterations of the high grade gliomas from which they derive.
Material and Methods: Genomic alterations of 11 glioma cell lines (U87MG, A172, H4, LN18, T98G, SF767, SW1088, SW1783, U373, U118 and GOS3) were screened by array-based Comparative genomic Hybridization (aCGH). Gene copy number alterations for EGFR, p53, CDKN2A and PTEN were confirmed by Multiplex ligation-dependent probe amplification (MLPA). Methylation of MGMT gene was tested by Methylation Specific (MS)-MLPA and MS-PCR. The presence of EGFRvIII mutation was tested by RT-PCR.
Results: Analyses of the copy number changes of 11 most commonly used glioma cell lines revealed higher frecquency of genomic losses than gains. Genomic losses were detected at 1p, 3p, 6q, 7p, 9p, 10q, 13q, 17q and 21q, while gains were detected at 4q, Chr.7, 10q, 19q and 22q. Frequent hemizygous or homozygous deletion was found at CDKN2A and PTEN loci in 9 and 5 out of 11 cell lines, respectively. Gain of *PDGFRA* was detected in 2 of the cell lines. Nor high-level amplification of EGFR, neither EGFRvIII mutation was detected in any of the cell lines. MS-MLPA and MS-PCR revealed MGMT gene methylation in all except one of cell lines.
Conclusions: Increased amplification and homozygous deletion occurrence was observed in cell lines of different sources such as breast, melenoma or lung tumours. However, in the case of the 11 glioma cell lines studied here, none of them carried the EGFR amplification, a frequent alteration in primary gliomas. Homozygous CDKN2A deletion was frequently observed in glioma cell lines, as occur in cell lines derived from other histologies and in glioma tumours. Crhomosome 7 gain and PTEN deletions represent the most specific glioma alteration present in the glioma cell lines.

478 Tetraploidy is more prominent in luminal than triple negative BRCA2 mutated breast tumours

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Background: Tetraploidy and aneuploidy can be caused by cell division errors and are frequently observed in many human carcinomas. We have recently reported delayed cytokinesis in primary human fibroblasts obtained from *BRCA2* mutation carriers, implying a function for the BRCA2 tumour suppressor in completion of cell division (Jonsdottir *et.al.* Cellular Oncology 2009; 31). Here, we address ploidy aberrations in breast tumours derived from *BRCA2* germline mutation carriers.
Material and Methods: Flow cytometry data on selected breast tumour samples (n = 236), previously screened for local *BRCA* mutations, were analyzed and ploidy aberrations compared between *BRCA2*-mutated (n = 71) and matched sporadic (n = 165) tumours. Differences in ploidy distribution were examined with respect to molecular classification, previously defined by immunohistochemistry on tissue microarray sections.
Results: No differences were found in the overall ploidy distribution between *BRCA2*-mutation carriers and non-carriers. However, tetraploidy was significantly 2.9 times more common in *BRCA2* breast tumours than sporadic. In *BRCA2* tumours, tetraploidy was associated with luminal characteristics whereas in sporadic cases, tetraploidy was more common in triple-negative tumours.
Conclusions: The increased frequency of tetraploidy in *BRCA2* associated tumours may be linked to cell division errors, particularly cytokinesis. Additionally, tetraploidy emerges predominantly in *BRCA2* breast tumours displaying luminal rather than triple-negative phenotypes.

479 The functional role of ADAM23 on SKMel-37 melanoma cells

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The ADAM23 (A Disintegrin And Metalloprotease) gene codes for a membrane-anchored glycoprotein with multiple domains, including an inactive metalloprotease domain and an active disintegrin domain that interacts with αvβ3 integrin. ADAM23 expression is reduced in different types of tumours and in breast tumours it is associated with the development of distant metastasis and a worse disease outcome. In MDA-MB-435 tumour cells, the silencing of ADAM23 gene is correlated with enhanced αvβ3 integrin activation and results in an increased cellular adhesion and migration. *In vivo* experiments with this cells showed that ablation of ADAM23 expression enhanced pulmonary tumour cell arrest in immunodeficient mice. In this work, we intend to explore the functional role of ADAM23 on the modulation of αvβ3 integrin activation using another cell model: the human melanoma SKMel-37 cell line.
The pAVU6+27 plasmid vector containing short hairpin RNA (shRNA) construct targeting ADAM23 was used to reduce ADAM23 expression in the SKMel-37 melanoma cells. Stable knockdown clones (SKMel-shRNA) were used in *in vivo* and *in vitro* experiments. The preliminary results showed that ADAM23 ablation modulated the activation of αvβ3 integrin in SKMel-37 cells. Moreover, the ablation of ADAM23 expression in these cells resulted in reduced proliferation when compared to control cells and enhanced invasion and migration *in vitro*. Additional functional assays are currently being conducted, including an *in vivo* spontaneous metastasis assay in immunodeficient mice to further investigate the role of ADAM23 in metastatic process.