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Altered NDRG1 expression in breast cell lines influences apoptosis

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Background: Several genes on chromosome 8q are amplified and show increased expression in breast tumours and breast cell lines. One of these genes, NDRG1, has been shown to be expressed at a higher level in cancerous than non-cancerous tissue of the same origin. However, increased NDRG1 expression has also been shown to inhibit tumour cell growth and NDRG1 has been suggested as a candidate metastasis suppressor gene in breast and prostate cancer. In addition, NDRG1 is induced in hypoxic conditions and results have suggested that in some cell lines NDRG1 expression is necessary for TP53-mediated apoptosis.

To gain more insight into the role of NDRG1 in tumour development, we have developed stable breast cell populations where the NDRG1 expression is reduced by RNAi and cell populations ectopically expressing the NDRG1 cDNA. In these studies we have selected cell lines with characteristics corresponding to the basal and luminal breast cancer subtypes.

Methods: We have used the two basal breast tumour models, SUM102 and ME16C2, which have high endogenous expression of NDRG1, and one luminal cancer subtype model, ZR-75-1, with low endogenous NDRG1 expression. Two shRNA constructs against independent target sequences on the NDRG1 transcript were introduced into the SUM102 and ME16C2 cell lines, resulting in a significant reduction in NDRG1 expression. The ectopic expression of the NDRG1 cDNA in ZR-75-1 gave a 100-fold increase in the NDRG1 protein level on western blots. Global gene expression analysis of the cell populations was also performed using microarrays.

Results: In the microarray experiments, SUM102 and ME16C2 cells transduced with the NDRG1 shRNAs were compared to cells transduced with empty vector. The differentially expressed genes identified suggested an enrichment of genes involved in cell adhesion and migration, which were generally increased in expression. Currently experiments are performed using the set of cell populations to investigate if NDRG1 has a role in cell migration and invasion. We have further treated the transduced cell populations with doxorubicin to induce TP53, and the results obtained by the TUNEL assay indicate that the induction of apoptosis is dependent of the level of NDRG1 expression. Experiments are also performed to assess how the cells with altered NDRG1 expression react to reduced oxygen levels ranging from hypoxic to anoxic conditions.

Conclusions: Our preliminary results indicate that NDRG1 is involved in cell migration and invasion. Our results further indicate that induction of apoptosis by doxorubicin is enhanced by increased levels of NDRG1 expression in the cell populations studied. This suggests that NDRG1 has a role in the apoptotic process both in the luminal and basal breast cancer cell models.

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Potentiation of antitumoral and antiangiogenic actions of docetaxel by docosahexaenoic acid (DHA): impact on micro- and macro-vascularization

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Tumour sensitivity to anticancer agents is a key feature for cancer curability. In previous studies, we reported that marine origin long chain polyunsaturated n-3 fatty acids such as docosahexaenoic acid (DHA) increased tumour sensitivity to anthracyclines, both in human breast cancer cell lines and in autochthonous rat mammary tumours (Germain 1998, Colas 2005). Furthermore we found that this dietary DHA effect was associated to a reduction in tumour vascularization. Thus, tumour vascularization might have a pivotal role in chemosensitization induced by DHA. Taxanes such as docetaxel are major drugs in breast cancer treatment. Since this chemotherapy displayed antiangiogenic properties, we examined whether dietary DHA potentiates its antitumor action through a vascular-based effect.

Autochthonous mammary tumours were induced in Sprague-Dawley female rats by N-methylnitrosourea. Then rats were assigned to a control group or to a group with a fish oil-enriched diet (with a supplementation in DHA 2.5%). When the largest tumour reached 2 cm², docetaxel (6 mg/kg) was injected weekly during 6 weeks. Subsequent changes in the tumour surface were evaluated. Using microbubbles of Sonovue® as a contrast agent in ultrasonography, we measured dynamic parameters of the whole vascularization (contrast diffusion speed, contrast transit time) and the macrovascularization was discriminated from the microvascularization by using a MATLAB® script.

Under docetaxel treatment, we observed a stagnation of tumour size in control rats whereas dietary fish oil induced tumour regression as early as three weeks of chemotherapy (-65% at the end of treatment). Despite unchanged dynamic parameters of vascularization, we observed in the control group an antiangiogenic effect of docetaxel (-45 %) using contrast-enhanced sonography. Furthermore, the data we obtained in DHA supplemented rats suggested an enhanced antiangiogenic effect of docetaxel on mammary tumour vascularization (-63 %). In fact, after discrimination of macro- and micro-vascularizations, we observed only a decrease in macrovascularization (-52 %) in control rats, whereas DHA supplemented animals showed a decrease in both micro- and macro-vascularizations (-43 % and -75 % respectively).

Thus reduction in both micro and macrovascularizations by DHA may account for the enhancement of docetaxel antiangiogenic properties and might contribute to the increase in docetaxel efficacy.

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Functional analysis of CDKN2A 5'UTR variants associated with family history of melanoma

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Malignant Melanoma derives from the neoplastic transformation of melanocytes and can arise de novo or from pre-existing benign nevi. Melanomas can occur in cancer prone families susceptible to a variety of cancers, such as in Li-Fraumeni syndrome families, or be the predominant cancer type in the case of Familial Cutaneous Malignant Melanoma (OMIM %155600). The CDKN2A gene is an established high-penetrance melanoma susceptibility gene. Germline CDKN2A mutations are observed in approximately 20–40% of melanoma-prone families from around the world. We have identified four Italian patients with established family history of melanoma that did not present mutations in the coding regions of CDKN2A (both p16 and p14arf were examined) nor of CDK4, but exhibited heterozygous variants in the 5'UTR of CDKN2A. To begin addressing the functional consequences of these novel variants we cloned the 271bp 5'UTR in different types of luciferase-based reporter vectors that can measure transcriptional as well as post-transcriptional effects. In a vector type, the UTR is cloned immediately upstream of the luciferase cDNA, which is transcribed from a minimal viral promoter. In a second vector the UTR sequence is placed upstream of a minimal promoter to evaluate its enhancer potential. We also constructed a bicistronic dual-luciferase vector that could test the effect of the variants on cap-independent translation.

To validate the reporter assays we examined a G>T transversion at the –34 position of the UTR that was previously identified in large melanoma-history family and shown to give rise to a novel AUG translation initiation codon out-of-frame with the canonical AUG, resulting in impaired translation of the gene. We also tested known single nucleotide polymorphisms (SNP) at the -33 and -191 positions. Unlike the two SNPs, the G-34T variant resulted in decreased activity of the luciferase reporters in the various plasmid types. Experiments are underway to examine the newly identified variants in these gene reporter assays. Quantitative PCR is also being used to further characterize the transcriptional/post-transcriptional effects of the variants. Finally, allele-specific qPCR is being attempted using lymphoblast cell lines derived from the heterozygous patients to confirm the impact of the 5'UTR variants at the endogenous gene.

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Tumor cell growth and metabolism at low growth factor and nutrient levels

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The tumor microenvironment is characterized by deficiencies in the supply of oxygen and nutrients as well as by an acidic pH. To understand how these parameters affect energy metabolism and growth of tumor cells requires a systematic analysis of their interactions. Towards this aim we cultivated two breast cancer cell lines representing different degrees of malignancy (MCF-7, MDA-MB231) in medium containing 1% FCS