suggest that *Arg* allele at codon 72 of p53 gene might affect the risk of ultraviolet-induced Basal Cell Carcinoma.

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Investigating the role of Smad4 in TGF-beta signaling using high density microarrays

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Transforming growth factor-beta is a multifunctional growth factor whose best-known function is to inhibit cell growth and suppress tumor formation. Loss of TGF-beta growth inhibition is one of the most common cellular events in the pathogenesis of human breast, pancreatic and colon cancers. TGF-beta signals through a heteromeric signaling complex consisting of Smad2, 3 and 4. Disruption of the Smad signaling complex often leads to tumor formation. We have used both 60-mer oligonucleotide and cDNA microarrays to investigate the role of Smad4 in the TGF-beta controlled transcription program in tumor cells. These high density DNA microarrays, generated using Agilent's SurePrint inkjet technology, were used to profile global transcriptional regulation in breast, colon and pancreatic Smad4-null tumor cell lines in response to TGF-beta. Data from both microarray types showed a high degree of correlation in demonstrating that TGF-beta induces transcriptional activation and repression of genes involved in signal transduction, cell adhesion and transcriptional regulation across the range of cell lines tested. Data from a number of studies is presented comparing expression profiles from Smad4-null tumor cell lines to those from either Smad4-transfected cell lines or normal cell lines. These data indicate that the composition of the Smad signaling complex controls the specificity of TGF-beta signaling.

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Polymorphic (CAG)n and (GGC)n in androgen receptor and breast and ovarian cancer risk in BRCA1/2 carriers and non-carriers

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Introduction. The androgen receptor (AR) is involved in the regulation of hormone-responsive genes and variation within the gene is hypothesized to play a role in breast and ovarian cancer susceptibility. We therefore examined whether *AR* repeat alleles modify cancer risk in *BRCA1* and *BRCA2* mutation carriers and familial breast and ovarian cases in comparison with age-matched control group.

Patients and Methods. Results were generated from 109 cases with mutation in BRCA1 and BRCA2, 60 first-degree familial cases without mutation within BRCA1/2 and 113 controls. Genomic DNA was PCR amplified using fluorescently labeled primers. The fragments were run on a 5% denaturing polyacrylamide gel, and amplicon length was determined relative to size standard by automated fluorescence detection. As in previous studies, $(CAG)_n$ repeat lengths of <22 were classified as short, and those of >=22 were classified as long. For $(GGC)_n$ repeats, those < 17 were classified as short, and those >= 17 were classified as long.

Results. Within the group of BRCA mutation carriers there was a significant difference in CAG cumulative repeat size between women with and without ovarian carcinoma (82.4% and 62.7% of CAG>=43, respectively, OR 2.78, $p<0.05$). GGC size was related to breast cancer presence: cumulative GGC>=45 was found in 33.3% of breast cancer cases and 57.6% of patients without breast cancer (OR 0.37, $p<0.05$). When the group of mutation carriers was compared to healthy subjects and familial breast cancer cases, there was no observed difference in CAG cumulative length, while a significant decrease in frequency of GGC cumulative >=33 was revealed: 45% in the group of mutation carriers vs. 67.4% in healthy subjects and 71.7% in familial breast cancer patients (OR 0.4 and 0.32, respectively; $p<0.005$). This study, one of the first to examine both $(CAG)_n$ and $(GGC)_n$, suggests a role of CAG long repeat for the development of ovarian cancer in BRCA mutation-carriers, while long GGC repeats seem to protect against breast cancer in these patients. In addition, our data show that long GGC repeat (>=17 repeats) is less common between breast and ovarian cancer cases when compared to general.

Conclusion. These results imply that CAG and especially GGC repeat length can potentially serve as a useful marker to identify a subset of individuals at higher risk of developing breast and ovarian cancer in BRCA1/2 mutation carries and familial cases.

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HER2 polymorphism and the risk of breast cancer

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Introduction: Breast cancer is a major public health problem around the world, and its carcinogenesis is not yet well understood. The Human Epidermal growth factor Receptor-2 (HER2) seems to play an important role in the development of this neoplasia, and genetic alterations in this gene, such as point mutations and polymorphisms have been detected in breast cancer patients. The aim of our study was to analyze the frequency of a single nucleotide polymorphism in the *HER2* gene in a southern European population.

Materials and Methods: The study included 161 patients who were diagnosed with breast cancer in the Portuguese Institute of Oncology Porto. DNA was extracted from peripheral blood of these patients. As control, the same experience was performed in blood samples from 142 healthy donors. DNA extracted from peripheral blood was submitted to Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP), in order to identify the possible *HER2* genotypes; Ile/Ile, Ile/Val and Val/Val. The restriction fragments were analyzed in a 3% agarose gel, stained with ethidium bromide.

Results: We found that the frequency of the Ile/Val genotype was higher in cases (39.1%) than in controls (24.0%), and the same was observed with the Val/Val genotype (4.4% and 2.8%, respectively). A twofold increase in risk of breast cancer was found among women who are carriers of a Val allele genotype Ile/Val and Val/Val genotypes (OR = 2.1; 95% CI: 1.3-3.4; p = 0.002).

Discussion: Our results indicate an association between the presence of the Val allele in the *HER2* polymorphism and the risk of breast cancer. Further studies are needed to evaluate the role of this polymorphism in the behavior of breast cancer.

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Reversible deposition of allele-specific primers by excess of complementary oligonucleotides drastically improves the reliability of allele-specific PCR

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Background: Allele-specific PCR (ASPCR) is considered to be a very straightforward approach for detection of single nucleotide polymorphisms (SNP), however its application remains somewhat limited due to insufficient reliability. Here we suggest a simple modification of ASPCR, that broadens the range of conditions in which ASPCR retains both high specificity and high sensitivity.

Material and methods: The idea of the method is based on the reversible deposition of allele-specific primers by addition of the corresponding complementary oligonucleotides. Since the bulk of the primers is diverted towards the excess of the competitor, DNA template has access to the primer only temporarily, when the latter is released from the depository duplex. Once annealing to the target sequence has occurred, the fate of the primer heavily depends on whether its 3' nucleotide matches or mismatches. In the case of match, even temporary hybridization to the DNA template is followed by immediate primer extension, due to residual activity of Taq polymerase in the annealing temperatures. Thus the matched primer becomes longer, and loses the ability to dissociate from the template. On the contrary, the extension of the 3' mismatched primer is compromised, thus increasing its chances to dissociate from the DNA template before the elongation occurs. Noticeably, the association/dissociation between allele-specific primer and its corresponding complementary oligonucleotide is absolutely reversible, because neither of the partners undergoes any modification. Therefore, despite the increased ASPCR specificity due to primer deposition, the absolute amount of allele-specific primer remains sufficient to support effective DNA template amplification even in the later stages of reaction.

Results: The suitability of this modification was proven using several examples of complicated ASPCR genotyping, such as TNF-alpha (G/A), DPD (G/A), XRCC1 (C/T), and CHEK-2 (C/T) allele discrimination. Conven-