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

602 POSTER

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.

604 POSTER

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.

605 POSTER

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-

tional ASPCR was either non-specific at all or showed specificity only in a very stringent conditions, which included low concentration of primers and magnesium chloride, high annealing temperature, and low number of PCR cycles; when any of the mentioned parameters was even slightly relaxed, non-correct genotyping occurred. However in the presence of the 3-fold excess of the depository oligonucleotide, ASPCR retained the specificity and reproducibility even if the PCR stringency was significantly reduced.

Conclusions: The deposition of allele-specific primers by complementary oligonucleotides evidently increased the reliability of ASPCR. The proposed modification may substantially facilitate SNP genotyping, either alone or in combination with other ASPCR improvements.

606 POSTER

Streptococcal preparation OK-432 is a new GMP-grade maturation factor of monocyte-derived dendritic cells

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Background: For vaccinations based on dendritic cells (DCs), maturation of DCs is important for the induction of effective T cell responses. A streptococcal preparation, OK-432, has been used as multi-cytokine inducer for management of cancer patients in Japan. We examined whether OK-432 can be a Good Manufacturing Practice (GMP)-grade maturation factor of DCs.

Material and methods: Immature monocyte-derived DCs (imDCs) generated from human peripheral blood mononuclear cells with granulocyte-macrophage colony stimulating factor and interleukin (IL)-4 were exposed to two types of common maturation factors, i.e., lipopolysaccharide and tumor necrosis factor-alpha plus prostaglandin E2, or OK-432 for another 2 days. Their surface expression of maturation-related molecules, allogeneic T cell proliferation, and cytokine secretion were analyzed with fluorescence-activated cell sorting (FACS), allogeneic mixed-lymphocyte reaction, and enzyme-linked immunosorbent assay, respectively. Activation of nuclear factor kappa B (NF- kappa B) was also examined with electrophoretic mobility shift assay.

Results: All agents examined increased both expression of maturation-related molecules such as HLA-DR, CD80, CD83, and CD86, and allogeneic T cell proliferation at a similar level in imDCs. Importantly, only OK-432 caused significant production of IL-12 p70 and interferon-gamma (IFN-gamma) at both the mRNA and protein levels. Induction of intracellular IL-12 and IFN- gamma in OK-432-stimulated DCs was also confirmed with FACS Calibur. Moreover, OK-432 induced activation of NF- kappa B in imDCs. Both cytokine secretion and NF- kappa B activation induced with OK-432 were suppressed when imDCs were pretreated with cytochalasin B, an inhibitor of endocytosis.

Conclusion: Our experimental data indicate that uptake of OK-432 by imDCs is an early critical event for secretion of both IL-12 p70 and IFN- gamma and that activation of NF- kappa B induced by OK-432 also contributes partially to these cytokine secretion. Since OK-432 is a GMP-grade agent, OK-432 may be a potential tool for vaccinations based on DCs.

607 POSTER

Induction of cytotoxic T lymphocytes that recognize a tumor-associated antigen, 90K/Mac-2 binding protein with an HLA-A2 restriction

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Background: 90K/Mac-2 binding protein (M2BP) is highly expressed in patients with various types of cancer and can influence the expression of surface molecules involved in immune responses on cultured cancer cells. We have reported that M2BP-specific immunity was observed in many lung cancer patients (Cancer 2002; 95: 1954-62). In this study, to identify HLA-A2-restricted immunogenic epitopes of M2BP, we generate cytotoxic T Lymphocytes specific for M2BP in vitro.

Materials & Methods: We selected 11 peptides (9-mer or 10-mer) derived from M2BP with an HLA-A*0201 binding motif according to peptide-motif scoring algorithms. M2BP-specific CTLs were generated from peripheral blood lymphocytes (PBLs) of HLA-A2-positive healthy donors by multiple stimulations of CD8-positive T lymphocytes with M2BP peptides. The induced CTL lines were examined for their specific responses to antigens by interferon-gamma production and standard ^{51}chromium-release assays.

Results: Three of the 11 CTL lines produced interferon-gamma in response to T2 cells (M2BP-/HLA-A2+) pulsed with the same peptide with a dose-dependent manner. However, only one CTL line induced using M2BP216-224 could lyse both peptide pulsed-T2 cells and a breast cancer cell line, MDA-MB-231 cells (M2BP+/HLA-A2+). The cytolysis was blocked by antibodies against HLA class I but not HLA class II molecules.

Conclusion: M2BP-specific CTLs could be generated in vitro using M2BP216-224 peptide. M2BP is expected to be useful as a target antigen in cancer immunotherapy.

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Streptococcal preparation ok-432 induces human dendritic cells maturation via up-regulation of toll-like receptors

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Dendritic cells (DCs) are potent antigen presenting cells to promote specific anti-tumor immune response. Streptococcal preparation OK-432 is supposed to induce innate immunity and up-regulation of toll-like receptors (TLRs). In the present study, we have investigated the effect of OK-432 on expression of TLRs as well as on maturation and activation of DCs in comparison with conventional tumor necrosis factor (TNF)-alpha. Human peripheral blood mononuclear cells (PBMC) were collected from five healthy volunteers and cultured in serum-free medium (AIM-V) in the presence of interleukin-4 (IL-4; 50ng/ml) and granulocyte-macrophage stimulating factor (GM-CSF; 50ng/ml) for 6 days. Then DCs were pulsed with tumor cell lysate obtained from human gastric cancer cell line MKN-45 for 12hr and further cultured for 48hr following addition of OK-432 (0.1 KE/ml). We compared it with addition of TNF-alpha (100 ng/ml) for DCs maturation. Cell surface phenotypes of DCs (HLA-ABC, HLA-DR, CD40, CD54, CD80, CD83 and CD86) were examined by flow cytometry, and cytotoxic T cell activity was evaluated using ^{51}Cr releasing assay. Expression of toll-like receptor (TLR)-4 and TLR9 after stimulation by OK-432, TNF-alpha or lipopolysaccharide (LPS) were examined using real-time reverse transcription polymerase chain reaction (RT-PCR). Expression of cell surface phenotypes examined was increased either on the surface of TNF or OK-432 treated DCs in a time dependent manner. No significant difference of the intensity of expression was noted between the two groups. Furthermore, ^{51}Cr releasing assay showed specific cytotoxity for MKN-45 with similar killing activity between the two groups. Expression of TLR-4 and TLR-9 were highest after LPS treatment, followed by OK-432 and TNF treatment, significantly higher in OK-432 treated group than in TNF treated group. The expression of TLRs peaked at 1 hr after stimulation in LPS and TNF, while it peaked at 2 hr after stimulation in OK-432. These results suggest that OK-432 has a potential role on human DCs for generation of CTL possibly via up-regulation of TLRs, and would offer an eligible protocol for human DCs in vivo immunotherapy especially for local administration.

609 POSTER

Tumour burden and interleukin-2 dose affect the synergism between low-dose total body irradiation and interleukin-2

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Background: Low-dose total body irradiation (LTBI) is believed to initiate various immune-mediated anti-tumour effects. We have previously shown a synergistic therapeutic effects when LTBI was used in combination with Interleukin-2 (IL-2) in a murine metastatic malignant melanoma model.

Aim of the work: To optimise the use of this combination treatment this study was performed to test the effect of tumour burden and dose of both LTBI and IL-2 on the therapeutic potential of this treatment strategy.

Material and Methods: Ten-week-old female C57BL/6 mice were inoculated i.v. (Day 0) with 1 million B16F1 malignant melanoma cells. The mice received either: no treatment, single fraction of LTBI alone, IL-2 treatment alone, or a combination of LTBI and IL-2. Two dose levels of LTBI and IL-2 were tested. LTBI was given either on day +7 or on day +10. IL-2 treatment was given over 5 days starting 24 hours after LTBI. Two days after the end of treatment, the mice were sacrificed and the lungs were removed and analyzed for tumor burden. Lung sections were also tested for tumor infiltrating cells using immuno-histochemical staining.

Results: LTBI (in the 2 tested dose levels), showed to independent therapeutic effects. IL-2 dose of (300.000 CU) that proved effective and