complex" (i.e., HLA class I antigens, tumor-associated antigens belonging to the cancer/testis antigens (CTA) class and accessory/co-stimulatory molecules) in neoplastic cells of different histotypes. These evidences strongly suggest that the extent of DNA methylation of cancer cells might favour tumor-escape from host's immune recognition, contributing to the reduced clinical efficacy of immunotherapeutic approaches for cancer treatment. In this scenario, the present study was designed to evaluate the immunomodulatory potential of new DNA hypomethylating agents (DHA) on neoplastic cells from solid tumors, aiming to identify novel strategies to improve the clinical response to cancer immunotherapies.

Materials and Methods: Cutaneous melanoma, mesothelioma, renal cell carcinoma and sarcoma cell lines were treated *in vitro* with the new DHA SGI-110, a dinucleotide of 5-aza-2′-deoxycytidine and guanosine. RT-PCR, quantitative RT-PCR and flow cytometric analyses were performed to investigate changes induced by treatment with SGI-110 in the constitutive immune profile of investigated cancer cells. CTA promoter methylation was evaluated by bisulfite sequencing.

Results: Treatment with SGI-110 induced/up-regulated the mRNA expression of a large panel of CTA (i.e., MAGE-A1, -A2, -A3, -A4, -A10, GAGE 1−2, GAGE 1−6, NY-ESO-1, SSX 1−5) in all investigated cell lines. Accordingly, exposure to SGI-110 up-regulated the constitutive expression of MAGE-A and NY-ESO-1 proteins, currently utilized as therapeutic targets in clinical trials of CTA-based cancer vaccination. Treatment with SGI-110 also up-regulated the expression of HLA class I antigens, HLA-A2 allospecificity and of the co-stimulatory molecule ICAM-1, in all investigated histotypes. Bisulfite sequencing analysis revealed a demethylation of MAGE-A3 promoter (-113/+130) following SGI-110 treatment of neoplastic cells, demonstrating a direct role of DNA methylation in the induction of this CTA.

Conclusions: These evidences strongly suggest that SGI-110 may represent an attractive therapeutic agent to comprehensively increase immunogenicity and immune recognition of neoplastic cells from solid tumors, and provide the scientific rationale for its clinical development to design new and possibly more effective chemo-immunotherapeutic approaches in patients with solid malignancies.

548 POSTER

Positive inter-regulation between beta-catenin and endothelin signaling in ovarian cancer cells: epigenetic regulation of ET-1 gene expression

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Although endothelin-1 (ET-1) and the endothelin A receptor (ET$_A$R) regulate different steps of ovarian cancer (OC) progression, the molecular mechanism controlling the expression of ET-1 in this tumor is unknown. We previously demonstrated that ET-1 activates β-catenin/TCF4 transcriptional activity promoting cell invasion and metastasis. Here, we show that ET-1 gene is directly regulated by β-catenin in OC cells. Thus, inhibition of β-catenin signalling results in lowered ET-1 promoter activity and expression, while enhanced β-catenin signalling leads to further activation of this gene. Chromatin immunoprecipitation (ChIP) demonstrated that, upon ET-1/ET$_A$R binding,β-catenin and its cognate DNA binding partner, TCF4, are recruited on the specific DNA element within the ET-1 promoter. Unravelling the role of the scaffold protein β-arrestin-1 as nuclear chaperone controlling β-catenin transcription activity, we demonstrated that β-arrestin-1 is required for ET-1 promoter activation, and for the recruitment of β-catenin on this promoter. In agreement with these findings, ET-1 promotes β-catenin and β-arrestin-1 interaction both in the cytoplasm and in the nucleus. Moreover, β-arrestin-1 siRNA leads to the loss of ET-1 mRNA expression and ET-1 secretion, as well as a significant inhibition of ET-1 promoter activity, thus suggesting the critical role of β-arrestin-1 in the β-catenin-dependent ET-1 gene expression. Further experiments showed that ET-1 promotes the nuclear association between p300 and β-arrestin and the recruitment of p300 on the ET-1 promoter, resulting in H3 and H4 histone acetylation and enhanced ET-1 expression. β-arrestin-1 represents a platform for achieving signal specificity that converges on β-catenin-mediated transcription of defined genes, such as cyclin D1 and matrix metalloprotease (MMP)-2, but not MMP-9. Moreover, ET$_A$R blockade with the specific ET$_A$R antagonist, zibontentan (ZD4054), abrogates both the engagement of β-arrestin and the interplay between ET-1 and the β-catenin in controlling gene transcription. Altogether these results reveal a positive inter-regulation between β-catenin and ET-1 that amplify the ET-1/ET$_A$R autocrine loop in ovarian cancer cells, in which β-arrestin-1 acts as a nuclear messenger mediating epigenetic mechanism in β-catenin-mediated ET-1 transcription.

Supported by AIRC.

549 POSTER

Unified carcinogenesis theory

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Transposable elements (TEs) are repressed in cells through DNA methylation during the gametogenesis and early embryogenesis. This process depends on some classes of small RNA such as PIWI-interacting RNA (piRNA), endogenous small interfering RNA (endo-siRNA) and microRNA (miRNA). We discovered recently that CG and CNG sites are found in cellular small RNAs more often than they should be found in random DNA sequence. Therefore we have hypothesized that binding of complementary DNA sequence by small RNA leads to recruiting of DNA methyltransferases that methylate *de novo* the DNA target. Apparently, primary strategy of cells at early development stages consists in total repression of any DNA sequences producing double-stranded RNA, since these sequences can be TEs.

Using TargetScan software, it was predicted that transcripts of great number of stage-specific genes are the targets of the small RNAs, especially miRNAs. Therefore, cell differentiation, starting with the earliest stages, should be accompanied with repression of genes of some small RNA, otherwise they would prevent expression of stage-specific genes. As a result, mechanism of RNAi-directed DNA methylation becomes inactive and cells can lose the repressive chromatin markers with time. This will excite the derepression of silent TEs and subsequent genome instability induced by them.

Firstly, TEs cause rearrangements in the miRNA gene loci. Because each miRNA affects the expression of many other genes, including miRNA genes, illegitimate activation of some miRNA genes leads to significant alteration in miRNA set and in epigenetic profile through the miRNA-dependent DNA methylation. Therefore, the cells begin to express a distorted gene profile that manifests as metaplasia and corresponds with pre-tumor status. Moreover, illegitimate miRNA expression can repress stage-specific genes directly and cells can lose the normal cytokine susceptibility. As a result, the course of cell differentiation proves to be complicated, requiring the high concentration of cytokines, or impossible at all.

Secondly, TE activity and epigenetic profile reorganization accelerate following evolution of the transforming cells during which these cells reactivate antiapoptotic genes and genes of angiogens and telomerase (or begin to use the ALT-mechanism through total demethylation of telomeres) as well as acquire the potential signs of cancer cells – ability for infiltrating growth and metastasis.

Thirdly, genome instability, caused through TE activity, can provoke illegal activation of cell oncogenes that initiate permanent cell proliferation and tumour formation. Nevertheless, in a number of cases epigenetic profile alteration can cause derepression of cell oncogenes independently.

550 POSTER

Airway-specific administration of the demethylating agent azacytidine effectively reexpresses tumor suppressor genes and inhibits lung cancer in mice

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Background: Hypermethylation of key tumor suppressor genes plays an important role in lung carcinogenesis. The purpose of this study is to explore the therapeutic potential of regional administration (via the airways) of the demethylating agent Azacytidine (Aza) for the treatment of early lung cancer.

Methods: We administered Aza solution directly into the trachea of ICR mice to study its toxicity and in nude mice bearing orthotopic human lung cancer xenografts to assess its antitumor activity. Aerosolized Aza was also given to mice with lung cancer induced by tobacco carcinogens.

Results: In vitro, Aza inhibited the growth of human lung cancer cell lines H226, H358, and H460 in a dose dependent manner. The concentrations to inhibit cell growth by 50% (IC50) were about 0.6−4.9 μg/ml. Aza reversed hypermethylation in the promoter of tumor suppressor gene RASSF1a in H226 cells at a 600-fold lower concentration than its IC50. In animal studies, intratracheal (IT) administration of 90 mg/kg Aza, the maximum tolerated dose of intravenously injected (IV) Aza, resulted in moderate pulmonary toxicity and 5-fold reduced myelosuppression compared with the same dose of IV Aza. Using an optimized multiple-dose schedule, IT Aza was about 3-fold more effective than IV Aza in prolonging the survival of mice bearing orthotopic H226, H358, or H460 xenografts, and did not cause any detectable toxicity. Using inhaled Aza at subtoxic concentrations, reexpression of tumor suppressor genes Rassf2 and Fhit in the bronchial epithelium has been observed as well as delay in tumor formation in a tobacco-induced carcinogenesis model.

Conclusions: At a non-toxic dose, Aza can reverse the hypermethylation in the human lung cancer cell lines and reexpresses tumor suppressor genes in mouse airway epithelium exposed to tobacco carcinogens. Regional administration to the airways enhances the therapeutic index of Aza by at least 75-fold. The potential of regional administration of Aza (including by aerosolization) for the treatment of advanced bronchial premalignancy deserves further investigation.

Pharmacogenetics

551 POSTER

Functional characteristics of human epidermal growth factor receptor (EGFR) polymorphisms

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Background: EGFR plays an important role in carcinogenesis and is an important target for cancer therapy. It is a trans-membrane protein with intrinsic tyrosine kinase activity which regulates signaling pathways that control cellular activities. Genetic alterations in *EGFR* have been implicated in a variety of human cancers, and have been shown to associate with variations in treatment outcomes mostly in Asians and Caucasians. We set out to systematically resequence the EGFR gene from African-American (AA) and Caucasian-American (CA) DNA samples and functionally characterize non-synonymous coding single nucleotide polymorphisms (cSNPs) observed.

Methods: All 28 *EGFR* exons, as well as splice junctions and portions of the 5′- and 3′- flanking regions were resequenced from 60 AA and 36 CA Coriell DNA samples. The non-synonymous *EGFR* constructs were transiently expressed in H727 lung cancer cells together with GFP to correct for transfection efficiency. A variety of functional parameters were measured.

Results: A total of 108 polymorphisms were observed, 3 of which were non-synonymous and changed encoded amino acids namely: Arg521Lys, His988Pro and Ser1162Asn. The His988Pro and Ser1162Asn were specific to the AA population and the Arg521Lys was observed in both populations. The 521Lys showed approximately 2.5 fold increase in levels of basal EGFR kinase enzyme activity and immunoreactive protein compared to either wild-type (WT), 988Pro or 1162Asn variant allozymes. The levels of enzyme activity and protein for 988Pro and 1162 Asn varaint allozymes however were similar to that of the WT. After 60 mins of stimulation in the presence of EGF ligand, phosphorylation (phosphor-EGFR) for the variant allozymes was reduced compared to the WT by 1.4-fold (988 Pro), 2-fold (1162Asn) and 2.5-fold (521Lys). Additionally, inhibitor characterization with erlotinib showed IC_{50} values ranging from 17.3 nM (521Lys), 41 nM (1162Asn), 46.3 nM (WT) to 65.8 nM (988 Pro). Cell proliferation in the presence of erlotinib was decreased for the 1162Asn and 521Lys variants compared to the WT and 988Pro variants.

Conclusions: The Lys521 polymorphism displayed increased basal kinase enzyme activity and protein levels but with higher inhibitor sensitivity and lower EGF stimulation compared to the 988Pro, 1162Asn and WT *EGFR* variants. These observations suggest that ethnic-specific pharmacogenetic variation in *EGFR* may contribute to efficacy and or toxicity during EGFR targeted therapy.

552 POSTER

CYP3A5*3 (rs776746) is associated with docetaxel-specific toxicities during adjuvant breast cancer chemotherapy

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Background: Systemic therapy is a commonly administered treatment modality in cancer care. Adverse drug reactions (ADR) during chemotherapy affect quality of life and may hinder delivery of adequate doses in a sub-population of patients, thereby limiting the therapeutic benefit. The heritable nature of CYP gene polymorphisms influencing drug metabolism and clearance are well characterized. In this study we investigated germ-line polymorphisms in several of CYP genes including CYP3A5 (CYP3A5*3; G>A, rs776746); and drug efflux pump, MDR1 (C 3435 T, rs1045642) for their association with drug-induced toxicity phenotypes.

Methods: DNA samples from breast cancer clinical trial patients (n = 184) receiving adjuvant poly-chemotherapy regimen with docetaxel (Taxotere), doxorubicin (Adriamycin) and cyclophosphamide in Edmonton, (Alberta, Canada) were selected for this study. All subjects signed an informed consent and the study was approved by institutional research ethics board. Following stringent data filtering criteria, a total of 147 cases were considered for association analysis. Patients with drug induced toxicity scores of 0−2 (n = 57) served as a reference (controls); these were compared with $\geqslant$ grade 3 toxicity groups: overall toxicity (group I, n = 90), docetaxel-specific (group II, n = 36) and non-docetaxel related (group III, n = 54). Hypersensitivity, fatigue, myalgia and neurotoxicity were classified docetaxel specific. Genotyping was performed using the Pyrosequencing technology platform. Associations between genotype and phenotype were analysed using unconditional logistic regression with SNPStats software.

Results: We identified statistically significant associations only for CYP3A5 among several CYP gene polymorphisms tested. Group I (p-value 0.013; Odds Ratio (OR) 4.19 (CI: 1.17–15.03) and II (p-value 0.015; OR 5.14 (CI: 1.26–20.92)) results were consistent with the predictions that docetaxel-related toxicities are mediated predominantly by CYP3A5*3 (heterozygote and variant genotypes) conferring risk. The MDR1 polymorphism was not associated with either overall or docetaxel-specific toxicity; however, CC genotype (wild type allele conferring higher expression, i.e., clearance of docetaxel) showed marginal protection (OR 0.30, p-value 0.037) in non-docetaxel related toxicity in group III.

Conclusions: Pharmacogenetic screening helps stratify patients to (i) identify groups at risk for chemo-toxicity and (ii) customize therapies to improve treatment outcomes.

553 POSTER

The influence of CYP2D6 and CYP3A5 pharmacogenetics on pharmacokinetics of tamoxifen and its metabolites in Asian breast cancer patients

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Background: To investigate the impact of *CYP3A5*3*, *CYP2D6*5* and **10* polymorphisms on the plasma concentrations and metabolic ratios of tamoxifen (TAM) and its three metabolites, N-desmethyltamoxifen (NDM), 4-hydroxytamoxifen (4OHT) and endoxifen (END).

Methods: A total of 165 Asian breast cancer patients were genotyped for *CYP3A5*3* and *CYP2D6* polymorphisms. Plasma levels of TAM and its metabolites were determined at steady state using HPLC with fluorescence detection. Genotypic-phenotypic associations between genotypes were performed using Kruskal-Wallis test and Mann-Whitney U-test.

Results: The END level [median (range)] of patients with *CYP2D6*10/*10* [8.03 (1.74–34.68)] was significantly lower compared to patients with *CYP2D6*1/*1* [19.55 (4.18–39.47), p < 0.001] or *CYP2D6*1/*10* [19.74 (7.26–33.24), p < 0.001]. Similarly, the NDM level of *CYP2D6*10/*10* carriers [374.41 (84.77–802.98)] was significantly higher than *CYP2D6*1/*1* [174.59 (40.82–448.65), p = 0.001] or *CYP2D6*1/*10* [279.43 (115.41–502.13), p = 0.006] carriers. Higher plasma concentration ratio of END/(TAM+NDM)$\times 10^{-2}$ (TMR_{NDM}) was observed in patients with **1/*1* genotype in comparison to patients with **1/*10* and **10/*10* genotypes [**1/*1* vs **1/*10* vs **10/*10*: 5.03 (2.94–7.45) vs 3.91 (0.98–7.24) vs 1.50 (0.51–10.69), P < 0.001]. Likewise, the plasma concentration ratio of END/(TAM+4OHT)$\times 10^{-2}$ (TMR_{4OHT}) ratio was found to be lower in **10/*10* carriers [4.16 (1.14–17.05)] compared to **1/*1* [11.05 (6.09–14.73), P < 0.001] and **1/*10* [9.18 (2.39–18.70), P < 0.001] carriers. Although *CYP2D6*5* was not significantly associated with plasma levels of the analytes, patients carrying the **5* allele was associated with lower TMR_{NDM} [**1/*1* vs **1/*5*: 5.03 (2.94–7.45) vs 3.30 (2.61–4.56), P = 0.001] and TMR_{4OHT} [**1/*1* vs **1/*5*: 11.05 (6.09–14.73) vs 8.49 (5.26–11.77), P = 0.030]. No association between *CYP3A5*3* polymorphism and plasma concentrations of tamoxifen and its metabolites or the TMRs was observed.

Conclusion: *CYP2D6*5* and *10* polymorphisms were found to influence the plasma concentration and metabolic ratios of TAM and its metabolites in this exploratory study. The functional impact of polymorphisms present in genes encoding other enzymes involved in tamoxifen biochemical pathway should be investigated.