Human Transcription Factors RT2 Profiler PCR Array (PAHS-075A − SABiosciences − Qiagen) was used to evaluate the profile of the expression of 84 transcription factors using real time PCR. The PCR array was validated through analysis of the expression levels of 8 genes by quantitative real time PCR using EVA Green dye.

Results: We analyzed transcription factors downstream of signaling from cytokines, chemokines and growth factors, signaling from androgen, B-cell, G-protein, T-cell and Toll-like receptors, and of signal transduction pathways like JAK/STAT, JNK, MAPK, NFkB, Notch and WNT. The SET profile showed 74 down-regulated and 7 up-regulated genes. Eight genes were validated (6 down-regulated and 2 up-regulated) in HEK293 cells overexpressing SET and confirmed in HN13 cells after SET knockdown.

Conclusions: SET accumulation in OSCC (previously validated by us) may control the expression of many transcription factors fundamental in the cell signaling and this could have implications in cancer progression and treatment.

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DNA methylation: an epigenetic pathway to cancer and a promising target for anticancer therapy in breast cancer

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Objective: To determine whether Estrogen Receptor 1(ESR1) (+) and ESR1(−) status relates to epigenetic changes in breast cancer-related genes and to correlate with molecular breast cancer subtypes.

Methods: Since January/02 to June/05,we quantified methylation levels ERS1 gene in serum of 92 pts breast cancer.A PCR quantitative technique was used to analyze levels of methylation gene.We also examined and correlationed the expression of ESR1 in tumors by immunohistochemistry with molecular phenotype.

Results: Median age was 58 years (32−88); 69% were postmenopausal women. Nodal involvement (N0; 63%, N1; 30%, N2; 7%),tumor size (T1; 58%, T2; 35%, T3; 4%,T4; 4%) and grade (G1;20%,G2;37%,G3;30%).Of the cases, 37pts (40%) were Luminal A (LA), 32 pts (33%) Luminal B (LB), 14 pts (15%) Triple-negative (TN) and 9 pts (10%) HER2+. The methylated ESR1 in serum was significantly associated with ESR1(−) in breast tumors >80%($p = 0.0179$). Methylation ESR1 was preferably associated with TN(80%) and HER2+(60%) subtype. Nevertheless unmethylation ESR1 was found more frequently in LA(71%) and LB(59%) phenotype. With a median follow up of 5 years, we found worse overall survival (OS) with more frequent ESR1 methylation gene($p > 0.05$), Luminal A;ESR1 Methylation OS at 5 years 81% vs 93% when was ESR1 Unmethylation. Luminal B;ESR1 Methylation 86% SG at 5 years vs 92% in Unmethylation ESR1. Triple negative; ESR1 Methylation SG at 5 years 75% vs 80% in unmethylation ESR1. HER2; ESR1 Methylation SG at 5 years was 66.7% vs 75% in unmethylation ESR1.

Conclusions: Gene promoter region hypermethylation is a significant event in primary breast cancer. However, its impact on tumor progression and potential predictive implications remain relatively unknown. Our study identifies the presence of variations in global levels of methylation promoters ESR1 genes in breast cancer with different phenotype classes and shows that these differences have clinical significance. Although numerous issues remain to be resolved,quantitative measurement of circulating methylated DNA may be of significance in the assessment and search of targeted therapy resistance related to ESR1 and HER2 status by epigenetic or transcriptional cancer therapy.

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Expression of methylthioadenosine phosphorylase (MTAP) in malignant pleural mesothelioma (MPM) and its implication for pemetrexed-based chemotherapy

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Introduction: The *MTAP* gene encodes for a key enzyme in the methionine salvage pathway. This gene is located at chromosomal locus 9p21, 100kb telomeric to *p16*, which is frequently deleted in malignant pleural mesothelioma (MPM). MTAP-deficient tumors are dependent on the *de novo* purine synthesis pathway, which is inhibited by drugs such as pemetrexed. This study investigates the MTAP expression in MPM and its relationship to pemetrexed-based therapy.

Material & Methods: DNA was extracted from tissue sections of paraffin-embedded tumor samples from MPM patients treated with pemetrexed-based therapy. Gene copy variation (GCV) of MTAP was determined using multiplex ligated PCR assay (MLPA), which included target probes for *MTAP* and *p16*. In addition, immunohisto- chemistry (IHC) was used to detect MTAP protein, using a validated monoclonal antibody. IHC was graded by two independent assessors using a composite score system which consisted of percentage score for positively stained areas multiplied by the intensity of staining, resulting in a score of 0–300. These findings were then correlated to tumor histopathology and clinical outcome, including disease control rates (DCR), median time to treatment failure (TTF) and overall survival (OS).

Results: Data for 52 MLPA and 59 IHC specimens were available for analysis. MTAP GCV was noted in 55% of samples. In all samples with MTAP deletion, there was co-deletion of *p16*. IHC score of 0–100 (null or minimal staining) and 101–300 (moderate to strong staining) were found in 55% and 45% of samples respectively. An association was observed between loss/minimal expression of MTAP protein and early disease stage ($p = 0.04$). The loss of MTAP gene or protein however, showed no significant association with DCR, TTF or OS.

Conclusion: Loss of MTAP protein expression was associated with early stage disease, but neither its gene copy status nor protein expression were predictive for clinical outcome in MPM patients treated with pemetrexed-based therapy.

	MTAP MLPA			MTAP IHC		
	+	−	p	+	−	p
DCR (%)	51	57	0.2	60	71	0.4
TTF (mths)	5.3	7.4	0.7	5.1	7.4	0.5
OS (mths)	6.2	10.3	0.9	10.0	10.1	0.7

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Promoter methylation of the RGC32 gene in non-small cell lung cancer and its clinical implications

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Background: Lung cancer is the leading cause of cancer-related deaths worldwide. Epigenetic inactivation of certain genes by aberrant promoter methylation is recognized as a crucial component in the initiation and progression of lung cancer. Response gene to complement 32 (*RGC32*) is formerly identified as a cell cycle regulator induced by activation of complements; however, its role in carcinogenesis is still controversial.

Methods: We have examined the methylation status in the promoter region of *RGC32* gene in non-small cell lung cancers (NSCLCs) using a methylation-specific PCR, and correlated the results with clinicopathological features.

Results: *RGC32* methylation was found in 45 of 173 NSCLCs (26.0%) and was related to the gene expression. *RGC32* methylation was more frequent in females than in males ($P < 0.05$). *RGC32* methylation was not significantly associated with the prognosis of patients; however, when the patients were categorized by *TP53* mutational status, the effect of *RGC32* methylation on prognosis was significantly different between those with and without *TP53* mutations ($P = 0.005$ [test for homogeneity]); specifically, *RGC32* methylation was associated with a significantly worse survival in the cases with wild-type *TP53*, whereas it exhibited a better survival outcome in the cases with *TP53* mutations.

Conclusion: The current findings suggest that methylation-associated down-regulation of *RGC32* plays an important role in the pathogenesis of NSCLC, particularly in females. However, further studies with a large number of cases are needed to confirm our findings.

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Immunomodulatory activity of SGI-110, a 5-aza-2′-deoxycytidine-containing demethylating dinucleotide

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Background: We have recently reported that aberrant DNA hypermethylation down-regulates the expression of components of the "tumor recognition 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.

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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_AR) 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_AR 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_AR blockade with the specific ET_AR 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_AR 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.

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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.