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

L. Rosanò[1], R. Cianfrocca[1], F. Spinella[1], V. Di Castro[1], P.G. Natali[1], A. Bagnato[1]. [1]*Regina Elena Cancer Institute, Experimental Research Center, Rome, Italy*

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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Unified carcinogenesis theory

V. Halytskiy[1]. [1]*Palladin Institute of Biochemistry National Academy of Sciences of Ukraine, Molecular Immunology Department, Kiev, Ukraine*

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.

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

Y. Zou[1], X. Qiu[1], A. Saxena[1], R. Perez-Soler[1]. [1]*Albert Einstein College of medicine, Medicine/Cancer Center Chanin 626, Bronx, USA*

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.