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Down-regulation of MET via DNA triplex-forming oligodeoxyribonucleotides targeting the promoter of c-Met gene results in growth inhibition and apoptosis in liver cancer

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Background: MET, the receptor for hepatocyte growth factor has been proven to play critical role during normal as well as malignant cell proliferation, cell survival and other critical cellular events. We have utilized transcriptional silencing via DNA triplex formation to selectively modulate MET expression.
Methods: Sequence specific TFOs were designed against *c-Met* promoter (TFO-1 from −142 to −119, 5′-AGGAGGGGGGAGAGG-3′ and TFO-2 from −644 to −620, 5′-AAGAAAAAAAGAAAAAAAAAAAG-3′). Antigene effect of TFOs was observed by western blot. Phospho-Kinase array was performed to analyze several other intracellular kinases in response to the TFOs. The efficacy of TFO was also observed in rat model system. Liver tumor was induced in male wistar rats by oral administration of diethylnitrosamine (40 ppm/day) for 8 weeks. Development of tumors was observed by Magnetic Resonance Imaging (MRI) and confirmed by histopathology. Test groups were treated with TFO (4 mg/kg) for 3 or 5 weeks respectively and MET expression and apoptotic activity were assessed.
Results: Interestingly, only TFO-1 treatment brought down MET levels by 50% with concomitant rise in pro-apoptotic proteins, Bax and p53 and decreased levels of anti-apoptotic protein, Bcl-xL. Observed loss of phosphorylation in ERK, MEK, AKT, Src, FAK, β-catenin, etc., indicate clearly the anti-proliferative effect of TFO-1. The regression of tumor volume by 90% as seen by MRI and 5 fold increase in apoptotic activity in association with MET down-regulation corroborates very well with *in vitro* data.
Conclusions: The results clearly point out that TFO-1 targeted MET leads to cell death via apoptotic pathway and therefore, DNA-triplex based therapeutic approaches hold promise in the treatment of malignancies associated with MET overexpression. The present study also throws light on the importance of MET targeting in late stage tumors as it is involved in the maintenance of the tumor and supports the hypothesis of 'oncogene addiction' by tumor cells.

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Evaluation of the role of mir-34b in modulation of radioresistance in non-small-cell lung cancer

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Radiotherapy is the major therapeutic weapons in lung cancer, allowing greater local control of disease and reducing the occurrence of metastasis. However, the resistance to radiotherapy is frequent, and involves molecular mechanisms still poorly understood. The microRNAs of the miR-34 family, miR-34a, miR-34b and miR-34c, described as effector molecules in the cellular response to activation of P53, have low expression levels in lung cancer. On the other hand, the mRNA of BCL-2, involved in apoptosis and autophagy, is among the targets of miR-34 family.
The aims of our study are to clarify the involvement of miR-34b over-expression in the modulation of radiation response in NSCLC (Non-Small-Cell-Lung-Cancer) cell lines and the mechanisms involved.
For these purposes we used two radioresistant NSCLC cell lines, the A549 cells, with an activating mutation in KRAS, and the H1299 cells, having a deletion of the P53 gene in homozygosity. The basal expression of miR-34 family members in the two cell lines was accessed by real time RT-PCR. Cells transfected with a precursor of pre-miR-34b or with a negative transfection control (75 nM) were submitted to different 99mTc irradiation doses exposure. The response to irradiation was assessed by cell survival curves obtained by clonogenic assay, by characterizing cell death by flow cytometry, using the double staining with annexin V and propidium iodide, and by the quantification of BCL-2, BAX and P53 protein expression levels, by flow cytometry using monoclonal antibodies labelled with fluorescent probes.
Our results show that both cell lines revealed low expression levels of miR-34 family members, more pronounced for miR-34b/c.
The over-expression of miR-34b, sensitize A549 cells especially to low doses of radiation (synergistic effect), in agreement with the observed decrease in cell survival. These results may be related with the decreased in BCL-2 expression and with the presence of wild type P53. In fact, in the H1299 cells, that have a delection in P53, the over-expression of miR-34b didn't influence the radiosensibility. On the other hand, in these cells we didn't observe a decrease in BCL-2 expression.
These results suggest that P53 may influence the response to radiotherapy in NSCLC that may be mediated by BCL-2 levels regulated by miRNA 34b.
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Fra-1 is an independent prognostic factor in esophageal squamous cell carcinoma and related to cell proliferation, migration and invasion in vitro

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Purpose: Fos related antigen 1(Fra-1) is a proto oncogene encoding a member of the activator protein 1 (AP-1) transcription factor. Fra-1 is activated in a variety of human tumors and gene ablation could suppress the invasive phenotypes of many tumor cell lines. The expression of Fra-1 involves tumor progression and invasion, and we investigated the significance of Fra-1 expression in esophageal squamous cell carcinoma (ESCC) by studying their protein expression and the effect of its down regulation on cell proliferation, motility and invasion.
Material and Methods: Surgical specimens from 164 patients with ESCC were evaluated immunohistochemically to investigate the expression of Fra-1. Fra-1 expression was compared among various clinicopathologic characteristics, and overall survival was analyzed. The rate and intensity of Fra-1 positive cells were also investigated. The role of Fra-1 in cell proliferation, motility and invasion was assessed by down regulation of Fra-1 expression using ESCC cell lines.
Results: Fra-1 expression was positive in 127 (77.4%) ESCC patients. Fra-1 protein was localized to the marginal areas of the ESCC tumors. Positive Fra-1 expression correlated with depth of tumor ($p < 0.0001$), lymph node metastasis ($p < 0.0001$), stage ($p < 0.0001$) and infiltrative grown pattern ($p = 0.0424$). A significant difference was seen in the survival rate between tumors with and without Fra-1 ($p < 0.0001$), and positive Fra-1 expression was revealed to be an independent factor related to poor prognosis. Metastastic lymph nodes with Fra-1 expression presented lower 5 year survival rates compared to lymph nodes negative for Fra-1 expression. After down regulation of Fra-1 expression, a significant decrease was observed in ESCC cells, in terms of cell proliferation, motility and invasion.
Conclusions: This study demonstrated patients of the Fra-1 positive group were associated with poorer prognosis compared to the negative group. Our findings also suggest that Fra-1 regulation may play an important role in the growth and invasion of ESCC.

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miR-205-mediated reversal of epithelial–mesenchymal transition modifies the drug sensitivity profile of prostate cancer cells

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Recent evidence indicates that tumor cells undergoing epithelial–mesenchymal transition (EMT) not only increase their metastatic potential but also become more resistant to drug, independent of the "classical" resistance mechanisms. In addition, it has been shown that residual tumor cell populations surviving after conventional drug treatments seem to be enriched for subpopulations of cells with mesenchymal features. We recently reported that miR-205, which is down-regulated in prostate cancer (PCa), is able to revert EMT in PCa cells [*Gandellini et al., Cancer Res 2009*]. In this study we proposed to investigate the ability of miR-205 to modulate the sensitivity of PCa cells to drugs with different mechanisms of action. The DU145 PCa cell line was stably transfected with specific vectors carrying the sequences of miR-205 and a control, and two polyclonal cell populations (DU145/miR-205 and DU145/miRVec) were selected for the study. Restoring the expression of miR-205 did not appreciably affect the growth potential of PCa cells. To test whether the basal level of miR-205 influenced the *in vitro* drug response, DU145/miR-205 and DU145/miRVec cells were analyzed for their clonogenic cell survival profiles after exposure to different concentrations of cisplatin. A dose-dependent reduction in cell survival was observed in both cell lines following cisplatin exposure, although DU145/miR-205 cells showed a significantly enhanced sensitivity to the drug compared to DU145/miRVec cells. Such a chemosensitizing effect was not associated to an increased apoptotic response following cisplatin exposure. However, in DU145/miR-205 cells an increased expression of autophagy-associated

markers, including Beclin 1 and ATG5, as well as a relocalization of LC3B protein, was observed, suggesting an enhanced propensity of the cells to undergo autophagic cell death as a determinant of their higher sensitivity to cisplatin. When we assessed the susceptibility of PCa cells to other chemotherapeutic agents, we observed an increased resistance of DU145/miR-205 cells to the mTOR inhibitor RAD001, whereas a comparable sensitivity to paclitaxel was observed for the two cell lines. Overall, these findings suggest that modulation of EMT in PCa cells may result in a different response as a function of the tested drug and that, only for selected agents, combination treatments including EMT-modulators, such as miR-205, can be envisaged to improve cell response.

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Newcastle disease virus Iraqi local isolate as a therapy for murine mammary adenocarcinoma: In vitro and in vivo study

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The avian paramyxovirus, Newcastle disease virus (NDV), is considered to be very promising. Since cytolytic strains of NDV possess a number of desirable properties in this regard. To evaluate the effectiveness of NDV Iraqi isolate (NDV-Iraqi Ahmed Nahi − IAN) as a tumor cytolytic agent, we have performed in vitro and in vivo experiments. In vitro tests studied oncolytic activity on different tumor cell lines by light and electron microscope. In vivo experiment using murine mammary adenocarcinoma allograft grown in mice. We compared antitumor activity of intratumoral injection of NDV-IAN to systemic intraperitonial treatment. In vitro results revealed necrosis and apoptosis induction. While in vivo results showed intratumoral treatment caused average of 92% growth inhibition ($p < 0.0001$), while intraperitonial treatment show 79% growth inhibition at the end of the experiment ($p < 0.0001$) compared to control group. Furthermore treatment groups showed prolong surviving. Histopathological pictures showed massive area of necrosis with infiltration of inflammatory cells mainly lymphocyte. Ultrastructural study showed budding of the virus from the treated tumor cells. Our results suggest that NDV Iraqi isolate (NDV-IAN) as a promising antitumor agent.

Genetics and epigenetics

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Potent in vitro and in vivo anti-tumor activity of ITF2357 by modulation of c-myc related miRNA signature in human Burkitt's lymphoma

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Background and Objectives: Recent studies support the existence of a c-*myc*–microRNA (miRNA) interaction within the genesis and the maintenance of Burkitt's lymphoma (BL). Myc oncoproteins have been found to inhibit the transcription of tumor suppressor genes by recruiting histone deacetylase (HDAC) proteins to target genes. We studied the in vitro and in vivo anti-tumor activity of a novel hydroxamate HDAC pan-inhibitor ITF2357 (Givinostat®, Italfarmaco S.p.A.) on BL cell lines with respect to its ability to modulate BL miRNA expression profile and c-myc target genes.
Methods: Standard MTT assay was used to define the half maximal cell-growth inhibitory concentration (IC_{50}) of ITF2357. Apoptosis and cell cycle phase distribution of treated and untreated cells were analysed by flow cytometry. MiRNA modulation was investigated by array analysis and real time PCR. Cell signalling proteins affected by ITF2357 treatment were analyzed by western blot, immunohistochemistry and confocal microscopy. In vivo anti-tumor activity of ITF2357 alone or in combination of cyclophosphamide (CTX) was studied in subcutaneous Raji xenografted SCID mice.
Results: Namalwa and Raji cell lines treated with 200 nM ITF2357 (48h IC_{50}) showed late and early apoptosis, with subG1 peak formation and G1 arrest respectively. To identify the molecular pathways affected by ITF2357, we investigated c-myc expression and NF-kB activation before and after treatment. Noteworthy, c-myc protein expression was reduced in treated BL cells while its mRNA levels did not change or even increased. As a possible mechanism impairing c-myc translation, we investigated the modulation of miRNA expression profile after treatment with ITF2357. Interestingly, in treated BL cell lines, let-7a and miR-26a that can negatively affect c-myc translation were up-regulated. According to recent evidences about the pro-apoptotic effects of NF-kB activation in human BL, we found that ITF2357 increased the acetylation of NF-kB subunit RelA and NF-kB nuclear localization in BL treated cell lines. The administration of 50 mg/kg ITF2357 to Raji xenografted SCID mice significantly reduced tumor growth compared to untreated control mice. Results from molecular analyses of the in vivo treated tumors were consistent with those obtained in in-vitro experiments. Finally, the combination of ITF2357 and CTX resulted more effective compared to CTX alone in completely eradicating the tumor in vivo.
Conclusion: The in vitro and in vivo anti-tumor effects of ITF2357 against BL cell lines were found associated with the reversion of crucial events in the *c-myc* driven lymphomagenesis, including the restoration of NF-kB activity and let-7a and miR-26a expression. The potent in vivo anti-tumor effects provided by the combined administration of ITF2357 and CTX might be translated in a novel and more effective therapeutic option for BL patients.

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Id1 enhances RING1b E3 ubiquitin ligase activity through the Mel-18/Bmi-1 polycomb group complex

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The helix–loop–helix inhibitor of differentiation and DNA binding (Id1) is well-known as an oncogene in various tumors. Although it has been reported that Id-1 promotes several oncogenic processes, it is still unclear whether Id1 functions through epigenetic transcriptional regulation. In this study, we examined the effect of Id1 on polycomb group (PcG) proteins, which are crucial epigenetic gene silencers, and found that Id1 regulated the expression of Mel-18 and Bmi-1, both of which belong to PRC1. We also confirmed that Id1 induced Mel-18 downregulation, which was mediated by the Akt pathway, and consequently upregulated the transcription of its target gene, c-Myc. Using a promoter-reporter, we demonstrated that Id1 regulated Bmi-1 transcription through c-Myc binding to its E-box in the promoter. Finally, we examined the activity of E3 ligase RING1b whose catalytic activity is increased by binding with the RING finger protein Bmi-1, and found that Id1 over-expression enhanced RING1b E3 ligase activity leading to accumulation of H2A ubiquitination and ubiquitin/proteasome-mediated degradation of geminin. Taken together, our study provided a novel link between Id1 and PcG proteins and suggested that Id1 may contribute to tumor development through PcG-mediated epigenetic regulation.

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Hydroxamate-tethered short chain fatty acid designer cancer prevention molecule

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Among various classes of histone deacetylase (HDAC) inhibitors, short-chain fatty acids exhibit the least potency, with IC(50) in the millimolar range. We rationalized that this weak potency was, in part, attributable to their inability to access the zinc cation in the HDAC active-site pocket, which is pivotal to the deacetylation catalysis. Based on the knowledge that the acetylation status of core histones plays a pivotal role in regulating gene transcription through the modulation of nucleosomal packaging of DNA. In a hypoacetylated state, nucleosomes are tightly compacted, resulting in transcriptional repression due to restricted access of transcriptional factors to their targeted DNA. Conversely, histone acetylation leads to relaxed nucleosomal structures, giving rise to a transcriptionally permissive chromatin state. The level of this posttranslational modification is maintained by a dynamic balance between the activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs), both of which are recruited to target genes in complexes with sequence-specific transcription activators. Aberrant regulation of this epigenetic marking system has been shown to cause inappropriate gene expression, a key event in the pathogenesis of many forms of cancer. For cancer prevention safety and low toxicity are of high importance for drug development. Hence, starting from butyric acid (present in dietary sources) however, concentrations are in milimolar range for this to be meaningful. Here we report an Hydroxamate-tethering approach wheer we explored the structural optimization of valproate, butyrate, phenylacetate, and phenylbutyrate by coupling them with Zn(2+)-chelating motifs (hydroxamic acid and o-phenylene diamine) through aromatic omega-amino acid linkers. This strategy has led to a novel class of Zn(2+)-chelating, motif-tethered, short-chain fatty acids that exhibited varying degrees of HDAC inhibitory potency. One hydroxamate-tethered