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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