concentration, showing IC50 ≈ 200 pM. Wound healing assays showed 35% of proliferation inhibition in B16F10 treated cells with 0,1nM of MkA09s at 6 and 12h of growing conditions. At the same time cell migration tests showed a 60% of inhibition in 6h of B16F10 cell line migration process, at a peptide concentration of 0,1 nM. At the same time, low toxicity of the peptide at mM concentration was confirmed using the MTT test. All presented results support that MkA09s peptide is capable to interfere in B16F10 cells adhesion and migration, acting as an integrin antagonist at nM concentration without apparent cellular toxicity. These authors suggest a possible anti-metastatic function for this cyclic peptide structure and specific in vivo experiments are the next step of our work to illuminate this question and to elucidate the specific action of the MkA09s peptide in other metastatic cell lines.
Grants: FAPERJ, CAPES.

## P1930

**Board Number: B489**

### Epigenetic silencing of β-catenin by NADPH oxidase-derived reactive oxygen species facilitates migration and invasion of HT29 colon cancer cells.

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β-Catenin, in one way maintains cell adherence making complex with other adherens junction molecules such as cadherins as well as other catenins, and in another way, being an integral part of the Wnt signaling pathway, it stimulates transcription of various genes important for cell growth. Previously, correlation of β-catenin promoter hypermethylation with loss of its protein expression, invasion/metastasis, and poor prognosis of non-small cell lung cancer is demonstrated. In our present study, we found decrease in β-catenin level and sustained production of reactive oxygen species correlated with 12-O-tetradecanoyl phorbol-13-acetate (TPA)-induced migration and invasion of HT29 human colorectal cancer cells. In contrast to β-catenin, TPA treatment did not alter E-cadherin and vimentin expression. In TPA-treated HT29 cells, DNA methyltransferase activity was increased, which was blocked by antioxidant (Vit. C), NADPH oxidase inhibitor (VAS2870), and DNA methylation inhibitor (5-azacytidine). TPA-induced decrease in β-catenin was blocked by pretreatment with NADPH oxidase inhibitors (Apocynin, diphenylene iodonium and VAS2870), NOX-2 siRNA, and 5-azacytidine, but not by histone deacetylase inhibitor (trichostatin A) and a proteasome inhibitor (MG-132). Furthermore, TPA-induced migration and invasion of HT29 cells were blocked by 5-azacytidine, but not by trichostatin A and MG-132. The results shed a light on epigenetic regulation of β-catenin by NADPH oxidase during colon cancer metastasis, which may serve as a new paradigm for cancer therapy.

## P1931

**Board Number: B500**

### shRNA Knockdown of NFKB1 expression inhibits proliferation and promotes apoptosis of renal cell carcinoma.

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Renal cell carcinoma (RCC) represents approximately 2-3 % of human malignancies. Despite all new therapeutic advances, almost all patients develop resistance to treatment and cure is rarely seen. The transcription factor KB (NFKB) comprises a family of transcription factors which has been associated with apoptosis resistance and progression of RCC. In this study, shRNA plasmid vector against NFKB1

gene was stably transduced into the Renca murine RCC cell line. Knockdown of NFKB1 was confirmed by quantitative real time PCR, Western blot and immunofluorescence analysis. The biological effects of decreased NFKB1 protein levels were evaluated, in vitro, by cell cycle and doubling time analysis and, in vivo, by tumor growth, cell proliferation (PCNA staining) and apoptosis (Caspase-3 staining) and necrosis (morphometry). The results revealed that NFKB1 knockdown efficiently inhibited the growth of Renca-shRNA cells in culture, induced cell cycle arrest at the G2/M phase and led to a significant decrease of the doubling-time. Moreover, NFKB1 shRNA vector suppressed tumor growth, enhanced apoptosis and necrosis compared with a wild type and mock control groups. In conclusion, our results suggest that specific silencing of NFKB is a potential therapeutic strategy for the treatment of RCC. This research was supported by FAPESP (2014/19265-6)

## P1932

**Board Number: B501**

### BIX02189 inhibits TGF-β1-induced lung cancer cell metastasis by directly targeting TGF-β type I receptor.

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Transforming growth factor-β1 (TGF-β1) promotes tumor metastasis by inducing an epithelial-to-mesenchymal transition (EMT) in cancer cells. In this study, we investigated the effects of BIX02189 and XMD8-92, pharmacologic inhibitors of the MEK5 [mitogen-activated protein kinase/extracellular-signal-regulated kinase (ERK)5] signaling pathway, on the EMT and migration of cancer cells induced by TGF-β1. In human A549 lung cancer cells, TGF-β1-induced EMT, cell motility, and expression of matrix metalloproteinase-2 were completely inhibited by BIX02189, but not by XMD8-92 or small interference RNAs specific to MEK5 and ERK5. Interestingly, BIX02189 strongly blocked the activation of TGF-β1 signaling components, and this inhibitory effect was not reproduced by MEK5 inhibition. Molecular docking simulation and kinase assays revealed that BIX02189 binds directly to the ATP-binding site of the TGF-β receptor type I (TβRI) and suppresses its kinase activity. Finally, the anti-metastatic effect of BIX02189 was validated in a TβRI-derived A549 xenograft mouse model. Collectively, these findings newly characterize BIX02189 as a potent inhibitor of TβRI that can block the tumor metastatic activity of TGF-β1.

## P1933

**Board Number: B502**

### Kaempferol Suppresses Transforming Growth Factor-β1-Induced Epithelial-to-Mesenchymal Transition and Migration of A549 Lung Cancer Cells by Inhibiting Akt1-Mediated Phosphorylation of Smad3 at Thr 179.

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Kaempferol, a natural dietary flavonoid, is well known to possess chemopreventive and therapeutic anticancer efficacy; however, its antimetastatic effects have not been mechanistically studied so far in any cancer model. This study was aimed to investigate the inhibitory effect and accompanying mechanisms of kaempferol on epithelial-to-mesenchymal transition (EMT) and cell migration induced by transforming growth factor-β1 (TGF-β1). In human A549 non–small lung cancer cells, kaempferol strongly blocked the enhancement of cell migration by TGF-β1–induced EMT through recovering the loss of E-cadherin and suppressing the induction of mesenchymal markers as well as the upregulation of