

promoter activity from constructs bearing these same mutations. We hypothesize that the ability of TMPyP4 to stabilize the chair-type quadruplex in the NHE III1 prohibits normal binding of this protein, and results in the decrease in c-MYC expression we have seen previously. A model is proposed, which explains how quadruplex formation occurs normally in the c-MYC promoter and regulates expression through the relative ability/inability of this protein to bind to the DNA.

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Additive/Synergistic interaction between MKP-1 inhibitors and anti-cancer drugs in human non-small-cell lung cancer cell H292

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A group of novel MKP-1 inhibitors developed in MAXIA has been shown to suppress tumor growth both *in vitro* and *in vivo* by activation of JNK and subsequent induction of apoptosis (see abstract by Allan Kaspar, et al.). The JNK pathway has been reported to be targeted by several marketed anti-cancer drugs such as cisplatin (CDDP), paclitaxel and adriamycin for their apoptosis induction. Therefore, it is rationale to hypothesize that inhibition of MKP-1, a downregulator of JNK activity, may enhance the chemotherapeutic effects of those antineoplastic agents that depend on the JNK pathway. This study investigated the combination therapy of MAXIA's MKP-1 inhibitor, MX7091, with cisplatin, paclitaxel and adriamycin. Apoptosis induction and general cytotoxic activity were evaluated in the non-small-cell lung cancer (NSCLC) cell line H292 by the cell death ELISA assay and the 3-4,5-dimethylthiazol-2-yl-2,5-diphenyl-tetrazolium bromide (MTT) assay, respectively. Concurrent exposure of H292 cells overnight with MX7091 (1 μ M) and cisplatin (10 μ M) led to an 11 fold increase of apoptosis whereas MX7091 alone only induced six-fold increase of apoptosis and cisplatin (10 μ M) alone did not significantly affect cell viability (1.4 fold). MTT assays revealed the similar observation in cell viability in both H292 cells and BxPC-3 cells (a pancreatic cancer cell line). Western blot analysis showed that JNK and c-Jun phosphorylation was synergistically enhanced by combined use of MX7091 and cisplatin as compared with each agent alone. These results suggest a synergistic interaction between MX7091 and cisplatin and are in agreement with a previous report that overexpression of MKP-1 can block the JNK activation and apoptosis by cisplatin. Paclitaxel (0.5 μ M) or adriamycin (0.5 μ M) alone induced apoptosis at 4.2 and 8.2 folds, respectively. Combination of MX7091 with paclitaxel or adriamycin resulted in 13.4 and 11.9 fold increase in cell apoptosis, respectively, indicating additive/synergistic interactions between MX7091 and those two drugs. More experiments are being performed to study the combination index (CI) of these drug combinations in different cancer cells. Animal studies are also being conducted to explore the *in vivo* synergism of the MKP-1 inhibitors and cisplatin and other anti-tumor agents.

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New differentiation-involution inducing agents for the treatment of breast cancer

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Breast cancer displays many properties that are exhibited during the developmental cycle of the mammary gland. The mammary gland comprises stromal and epithelial cells that communicate through an extracellular matrix (ECM) to control the function of the gland. The stromal cells control the ECM composition and its network structure in the mammary gland. Upon hormonal stimulation during pregnancy and lactation, stromal adipocyte dedifferentiate into preadipocyte causing a change in the ECM content and signal the ductal epithelial cells to proliferate. Once lactation ceases, stromal preadipocytes redifferentiate into adipocytes and change the ECM content such that the proliferating epithelial cells to undergo apoptosis and the mammary gland to remodel back to its resting or lead to adult nulliparous state. This process is called involution. An imbalance or incomplete involution can lead to breast cancer. Existing breast cancer drugs such as anti-hormone, and apoptosis inducing agents, act mainly on epithelial cells. MAXIA developed a new class of anti-breast cancer agents that mimic involution and function through stromal fat cells and the ECM. These compounds induce differentiation of preadipocytes into adipocytes and downregulate integrins, cadherins and Wnts. The changes caused by our compounds induce growth arrest and apoptosis of breast cancer cells and lead to tumor regression and prevention *in vivo*. Western blot analysis revealed that cylin D1, a down stream target of the integrin, cadherin and

Wnt signaling pathways, is also down regulated. The compounds showed additive/synergistic activity in the *in vivo* model with the anti-estrogen Tamoxifen. Thus, we have discovered a new class of differentiation/involution inducers that promise a new effective treatment for breast cancer when used alone or in combination with anti-hormonal therapies.

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Novel inhibitors of MKP-1 have potent anti-cancer activity *in vivo*

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The mitogen-activated protein kinase phosphatases (MKPs) are a subfamily of dual-specificity phosphatases that are capable of dephosphorylating and inactivating members of the mitogen-activated protein kinase (MAPK) family. Activation of c-Jun N-terminal kinase (JNK), a member of the MAPK family, has been shown to be involved in mediating apoptotic cell death. JNK activity is negatively regulated by MKP-1, which has been shown to dephosphorylate JNK and to protect cells from certain apoptotic stimuli. Additionally, MKP-1 overexpression has been observed in patients with prostate, ovarian, and lung cancers. MAXIA has developed small molecule inhibitors of the phosphatase MKP-1, MX7091 and related analogues, which induces JNK-activation and apoptotic death of tumor cells. *In vitro* phosphatase assays show that MX7091 and related compounds selectively inhibit MKP-1 activity. Treatment of cancer cell lines with nanomolar concentrations of MX7091 results in JNK activation, caspase activation, and apoptotic cell death. Additionally, MX7091 synergizes with the chemotherapeutic cisplatin to induce apoptosis of tumor cells. MX7091 efficacy correlates with overexpression of MKP-1 in tumor cells, consistent with MKP-1's role in maintaining cell survival. MX7091 significantly reduces tumor size and induces tumor remission, while increasing animal survival time in *in vivo* models of pancreatic, colon, and non-small cell lung cancer. These studies demonstrate that MAXIA's MKP-1 inhibitors represent a novel class of anti-tumor agents with potential clinical utility.

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alpha2-6-sialylated neolacto-series gangliosides serve as receptors for the anticancer drug rViscumin

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rViscumin is a heterodimeric cytotoxic plant protein currently in clinical phase 1 trials. Like ricin and other type II ribosome inactivating proteins (RIP) rViscumin consists of an enzymatically active A-chain being responsible for the toxicity towards tumor cells through inactivation of the translational machinery of the cell and a B-chain with carbohydrate binding activity. The B-chain is responsible for binding to the surface of the target cells, subsequently leading to internalisation. In this study we set out to investigate potential differences in the carbohydrate specificity of rViscumin and ricin which could explain the good tolerability and efficacy of rViscumin during preclinical and clinical development. In recent literature rViscumin as well as ricin are described as galactose-specific carbohydrate binding proteins. Employing solid phase binding assays rViscumin was shown to preferentially bind to terminally alpha2-6-sialylated neolacto-series gangliosides IV6Neu5Ac-nLc4Cer, VI6Neu5Ac-nLc6Cer, and VII6Neu5Ac-nLc8Cer. Only marginal binding of rViscumin to galactose-terminated neutral glycosphingolipids was determined, whereas reinvestigation of ricin specificity demonstrated this type II RIP as galactose-binding protein. In cytotoxicity assays with human promyelotic HL-60 cells and human bladder carcinoma 5637 cells IC₅₀ values of 1.16 μ M and of 12.1 μ M rViscumin were determined, respectively. CHO-K1 cells were resistant to rViscumin treatment up to a concentration of 5.26 nM tested. A direct correlation of rViscumin cytotoxicity and the expression of the receptor ganglioside IV6Neu5Ac-nLc4Cer was shown by means of a specific anti-Neu5Acalpha2-6Galbeta1-4GlcNAc-R antibody. The data revealed 3.7x10⁶ and 1.5x10⁶ receptor molecules per HL-60 and 5637 cell, respectively. CHO-K1 cells were negative, lacking alpha2-6-sialylated gangliosides. Moreover, CHO-K1 cells were rendered susceptible towards rViscumin cytotoxicity after exogenous application of human granulocyte gangliosides (HGG) which contain predominantly alpha2-6-sialylated gangliosides. From these data we conclude that rViscumin in contrast to ricin has