Taken together, our results suggest that MNTX activation of RPTP mu activity is central in inhibiting VEGF-induced Src and RhoA activation, rhoGAP inactivation, and angiogenesis. Our results indicate that MNTX may represent a potential therapeutic agent for the treatment of tumor-associated angiogenesis.

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Inducing apoptosis in colorectal tumor cells through inhibition of Aurora B kinase

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Background: Aurora kinases are serin/threonin kinases with oncogene potential. The overexpression of Aurora kinases was found in colorectal tumors and correlates with poor clinic prognosis. Recent studies showed that inhibition of aurora kinases inhibited the growth of colorectal tumors in vitro and in vivo. However, the underlying mechanism is still unknown. In this study, we examined the anti-tumoral signaling of aurora kinase inhibitor (AKI), ZM 447439.
Methods and Materials: The apoptosis and cells death were counted in Colo-205 and HCT-116^{wt} colorectal tumor cells, at different time points after administration of ZM447439, using Hoechst33342/propidium iodide double staining. Mitochondrial potential (TMRE fluorescence) and polyploidy formation (propidium iodide fluorescence) was examined using flow cytometry. Western-blotting was performed to assess phosphorylated histone H3 (Ser 10) and cleavage of caspases. To analyze the signal pathway of the Aurora kinase inhibitor, all assays were also undertaken in HCT-116$^{p53-/-}$, HCT-116$^{p21-/-}$, HCT-116$^{Bak-/-}$, HCT-116$^{Bax-/-}$ and HCT-116$^{Bak/Bax-/-}$ cells.
Results: ZM447439 inhibited phosphorylation of histone H3 (Ser 10), verifying its inhibitory capability to Aurora kinase. Inhibition of Aurora kinases with ZM447439 significantly stimulated increase of apoptosis and cell death in Colo-205 and HCT-116^{wt} cells in a concentration- and time-dependent manner. PARP was processed in HCT-116^{wt} cells treated with ZM447439. Knockout of p53, Bak, Bax or Bak/Bax in HCT-116 cells significantly reduced apoptosis and cell death induced by ZM447439, compared with wild-type ($p < 0.01$). A progressive dissipation of mitochondrial membrane potential was observed in HCT-116^{wt} cells 24 h (5.3%), 48 h (34.3%) and 72 h (58.3%) after treatment of 5μM ZM447439, normalized to control group respectively ($p < 0.01$). Knockout of p53, Bak, Bax or Bak/Bax in HCT-116 significantly protected cells from dissipation of mitochondrial membrane potential induced by ZM447439. Knockout of Bak, Bax or both Bak and Bax caused similar resistance to Aurora kinase inhibitor, while knockout of Bak and Bax showed a nonsignificant increase of the resistance. ZM447439 induced notable endoreduplication and polyploidy formation in all examined cell lines ($p < 0.01$), while HCT-116$^{p21-/-}$ was the most sensitive to undergo endoreduplication and form polyploidy.
Conclusion: The small molecular Aurora kinase inhibitor induced apoptosis and cell death in colorectal tumor cells in vitro and may be a new class of potential therapeutic agent for colorectal tumors. P53, Bak and Bax play important roles in Aurora kinase inhibitor induced apoptosis and cell death. Understanding the underlying mechanism may help to design new therapeutic concept.

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Celecoxib-induced apoptosis depends on Bak and is only marginally modified by Bcl-2 overexpression

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Background: Celecoxib and other selective cyclooxygenase-2 (COX-2) inhibitors are potent inducers of apoptosis and increase the efficacy of ionising radiation. Celecoxib induces apoptosis via a novel mitochondrial pathway requiring Apaf-1 and caspase-9. However, apoptosis induction is independent from expression level of anti-apoptotic Bcl-2 as demonstrated in our earlier investigations. We therefore wanted to evaluate the relevance of both crucial pro-apoptotic Bcl-2 proteins – Bak and Bax – for celecoxib-induced apoptosis in Jurkat T-Lymphoma cells.
Material and Methods: Induction of apoptosis was determined using COX-2- and Bax-negative Jurkat cell clones being (a) deficient for Bak (Jurkat Bak negative), (b) proficient for Bak (Jurkat Bak positive), or (c) proficient for Bak and overexpressing Bcl-2 (Jurkat Bcl-2). After treatment with 0, 50, 75 and 100 μM of celecoxib apoptosis was determined by flow cytometry (morphology, depolarisation of the mitochondrial membrane potential and nuclear fragmentation), fluorescence microscopy (chromatin condensation, nuclear fragmentation) and Western Blotting (caspase-activation, Bcl-protein-expression).
Results: Celecoxib induced substantial apoptosis in Jurkat cells expressing Bak (but not Bax). The extent of apoptosis was negligibly modified by overexpression of Bcl-2 in concordance with our earlier findings. But, apoptotis decreased substantially in Jurkat Bak negative cells lacking both proapoptotic multidomain Bcl-2 proteins, Bax and Bak. The apoptotic rates were clearly distinguishable from Bak positive cells even after increase of incubation time or concentration of celecoxib. Similarly, depolarisation of the mitochondrial membrane potential, activation of caspases-9, -8 and -3 as well as cleavage of the caspase-3 substrate PARP were only observed for Bak-positive Jurkat cells independent from Bcl-2 overexpression. In contrast, Bak-deficiency was sufficient to inhibit apoptotic changes.
Conclusions: Lack of Bak was sufficient to abrogate celecoxib-induced mitochondrial damage, caspase-activation and nuclear fragmentation. Anti-apoptotic Bcl-2 overexpression did marginally modify response to celecoxib. These data provide evidence for a Bak-dependent but COX-2-independent pro-apoptotic effect of celecoxib in Bax-negative Jurkat T-lymphoma cells in vitro.

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Serum matrix metalloproteinase 1 (MMP1) as a prognostic marker in bone metastases (BM) treated with bisphosphonates

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Background: Pre-clinical studies suggest that cancer cells in bone do express MMPs with collagenolytic activity (J Bone and Miner Res 2003;18:859−67). In human BM sections we found an higher expression of MMP1 in patients already treated with BP (ASCO 2002 abstract 1793). In this prospective study we determined whether the baseline activity of MMP1 was predictive of OS and SREs as defined by: pathologic fractures (PF), radiation to treat BM (RT) or spinal cord compression (SCC).
Methods: We studied 116 BM patients, median age: 64 years; 67% females; 61% breast cancer; 19% prostate cancer; and 20% other tumor types. All patients were classified according to x-ray pattern (lytic 54%, blastic 23%, or mixed 21%) and extent of BM (number of skeletal segments involved). At the time of study entry, 108 patients had serum levels of the MMP1 active form measured with enzyme
linked fluorescence immunoassay using reagents obtained from R&D Systems (limit of detection: 0.39 ng/mL). During the time period on study, patients received treatment with IV zoledronate (57%), IV pamidronate (28%), or more than one BP.
The proportional hazards model was used to investigate the correlation of MMP1 baseline level with OS and time to first SRE (TTSRE); and Poisson regression with the skeletal morbidity rate (SMR): number of SREs/person/year.
Results: The median follow-up was 21 months. The median value of MMP1 was 4.73 ng/mL (range: 0−32.3 ng/mL). During the time period on study, 38% had PF, 57% had RT, and 9.5% had SCC. Median TTSRE was 20 months and the SMR was 0.84. Median OS time was 29 months. Serum MMP1 levels were associated with increased mortality risk with a hazard ratio (HR) of 1.08, 95%CI 1.02−1.14, $p = 0.007$ (8% increased risk of death for each 1 ng/ML increase in serum MMP1). The correlation of MMP1 with OS was independent of the extent of BM (interaction test for MMP1 and the number of skeletal segments involved with bone metastases: $p = 0.8$). MMP1 serum levels were not associated with TTSRE (HR 1.01, 95%CI 0.94−1.08, $p = 0.64$) or SMR (HR 1.02, 95%CI 0.95−1.09, $p = 0.68$).
Conclusions: Serum levels of the active form of MMP1 at baseline is associated with decreased survival in BM patients on BP therapy. These results suggest that MMP1 might be a new target for the treatment of BM.