Targeting p90^{rsk} for the development of novel anticancer therapies

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Background: p90 ribosomal S6 kinases (RSK or p90^{rsk}) is a family of serine/threonine protein kinases involved in signal transduction that are activated by the MAPK/ERK pathway. Several studies have further suggested the importance of p90RSK dysregulation in cancer development and progression. Flavonoids of the kaempferol family, like SL0101, have been shown to act as inhibitors against members of the RSK family with a potential anticancer activity.
In this regard, we have investigated the potential antitumor activity of a semisynthetic kaempferol glucoside derivative named T*ac*, We further investigated whether this agent could suspend the mitogenic function of MAPK signaling pathway in colorectal cancer via RSK inhibition, its mechanism of action and we finally evaluated its antitumor activity in a human colorectal tumor xenograft/immunodeficient mouse model.
Materials and Methods: The *in vitro* antiproliferative effect of *Tac* against human cancer cell lines was examined using the SRB assay. COMPARE algorithm was further employed for an initial evaluation of the mechanism of action. *Tac*-induced cell death perturbations on cell cycle were examined on the HCT116 human colon cancer cell line using FACS analysis. Western blot was utilized for the detection of caspase activation, PARP cleavage and changes in the levels of MARCKS, p-MARCKS, ERK2, p-ERK1/2, RSK, p-RSK, total EF2, eEf2 and p-eEf2. Finally the antitumor activity of *Tac* was evaluated *in vivo* against HCT116 xenografts.
Results: COMPARE analysis revealed great similarities of the mechanism of action of *Tac* with DNA damaging agents. FACS analysis with PI and BrdUdr stain indicated that *Tac* treatment resulted in a delay in the transition from G2- to M-phase, subsequent blockage of the transition from G1- to S phase, and apoptosis through caspase activation. Under these specific experimental conditions *Tac* did not affect MARKCS or ERK1/2 protein levels and phosphorylation state but did alter RSK's activity as this was depicted by the eEF2 phosphorylation levels. Intraperitoneal (ip) administration of *Tac* at the maximum tolerated dose (MTD) significantly suppressed growth of HCT116 colorectal tumors in xenografts.
Conclusions: Under the experimental conditions tested herein *Tac* induced cell cycle arrest and apoptotic cell death in human colon cancer cells by a mechanism involving MAPK pathway and more specifically RSK inhibition. COMPARE analysis further suggested that *Tac* may act as a DNA damaging agent thus linking RSK to DNA damage. Finally for the first time we show that an RSK inhibitor may suppress the growth of human colorectal tumors in xenografts. Taken together, the *in vitro* and *in vivo* results suggest that RSK may be an important target for the development of new anticancer therapies.