249 DNA adduct recognition by proteins: identification, binding specificity and molecular consequences for S23906−1/DNA adduct

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Background: S23906−1 is an acronycine derivative presenting DNA alkylation properties and being original for its covalent bonding to the ***minor groove of the DNA*** leading to a ***local opening of the double helix***. Based on high antitumour activities, S23906−1 entered clinical trial in 2006–2007. However, the exact mechanism by which S23906−1 achieves its anti-tumour activity is not fully known. Interaction of proteins to DNA adduct is well known for cisplatin and ET-743 which are recognized by HMG-B1 and the nucleotide excision repair protein XPG, respectively, increasing cell death potency. Therefore, we wanted to identify the proteins that could interact with S23906/DNA adduct and understand the cellular consequences of such recognition.
Methods and Results: Using a proteomic approach we have identified HMG-B1 and GAPDH as proteins interacting with S23906-alkylated DNA. Direct binding of GAPDH, but not HMG-B1, to the S23906−1/DNA adduct was validated using EMSA. Such GAPDH interaction depends both on adduct and DNA sequence specificities. Indeed, GAPDH also recognizes Saframycin A (SafA) but not ET-743 adducts, both being DNA minor groove alkylating agents. GAPDH and S23906 are known to bind to both double- (ds-) and single-stranded (ss-) DNA. We showed that GAPDH interacts strongly with both S23906-alkylated ds- and ss-DNA.
Only little is known about the nuclear roles of GAPDH (DNA repair, transcription and replication) and its DNA binding potency in particular. To identify the possible consensus for GAPDH/DNA interaction we used a CASTing method to select the best recognition sequences from random oligonucleotides.
At the cellular level, it was already observed a nuclear translocation of the GAPDH followed by cell apoptosis, under several stresses (heat shock, H_2O_2, SafA treatment...). However, even if siRNA and cytotoxicity approaches showed a small effect of cellular GAPDH in S23906−1 cytotoxic effect, no nuclear translocation of GAPDH was observed after S23906 cell treatment by contrast with published results that used SafA.
Conclusion: GAPDH binds to S23906−1/DNA adduct in some specific way. The possible sequence-specificity of GAPDH to DNA will be discussed. GAPDH may be implicated in S23906−1 cytotoxicity through its role in DNA repair processes.

250 Small molecule inhibitors of cellular stress response pathways can modulate viability of malignant melanoma cells

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Background: The advanced metastatic form of malignant melanoma is usually refractory to all available treatments and has very poor prognosis. The 5-year survival rate has been estimated at only 6%, with median survival time of 6 months.
New molecular targets suitable for the development of more efficient therapies could be found through better understanding of the biology of malignant melanoma cells.
Constitutive activation of the extracellular signal-regulated kinase (ERK) pathway by somatic mutations of *N-Ras* and *B-RAF* proto-oncogenes seems to be critically important for melanoma development and survival, and a number of small molecule inhibitors of this pathway have already entered clinical trials. In contrast to the ERK pathway, the role of other mitogen-activated protein kinase (MAPK) pathways in melanoma cell biology still remains unclear.
Material and Methods: We have tested, in viability and proliferation assays, the response of several melanoma cell lines to SB202190, a small molecule inhibitor of the p38α/β MAPK pathways. At the same time, the morphology of cells was studied using light and electron microscopy. In addition to that, the effects of combined treatments with p38 inhibitors and small molecule inhibitors of other cellular signaling pathways were also tested.
Results: On its own, the pharmacological inhibition of the p38 MAPK had little impact on the viability of malignant melanoma cells. However, we have observed a strong induction of autophagy in p38 inhibitor-treated cells. Autophagy is a coordinated dynamic process of cytoplasmic material degradation in lysosomes which can promote both cell survival and cell death. Importantly, a simultaneous inhibition of autophagy using chemical inhibitors 3-methyladenine or Bafilomycin A1 significantly decreased the viability of several SB202190-treated melanoma cell lines, suggesting that autophagy might be required for the survival of melanoma cells that are lacking active p38 MAPK. In addition to that, p38 inhibitor-treated cells became extremely sensitive to thapsigargin, a small molecule inhibitor of endoplasmic reticulum (ER) calcium pump and ER stress inducer. These results indicate that p38 MAPK signalling might be necessary for the proper response of melanoma cells to ER stress.
Conclusions: Taken together, our results suggest that p38 MAPK signaling can play an important role in the biology of melanoma cells and together with the ER stress response and autophagy pathways might be a suitable target for the development of novel therapeutic strategies for malignant melanoma.
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251 Potent and efficient testosterone suppression by chronic administration of novel metastin analogues, TAK-448 and TAK-683, in male rats

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Background: Metastin/kisspeptin is the cognate ligand for a G-protein-coupled receptor, GPR54, and functions as a key regulator in the control of hypothalamic gonadotoropin-releasing hormone (GnRH) neurons. TAK-448 and TAK-683 are metastin analogues. We evaluated their *in vitro* receptor activities as well as the effect of chronic administration on plasma testosterone (T) levels, weights of genital organs, and pituitary and GnRH neuronal responsiveness using male rats.
Material and Methods: Agonistic or receptor-binding activities of TAK-448 and TAK-683 were evaluated using GPR54-expressing Chinese hamster ovary cells or their membrane fraction. For *in vivo* studies these compounds were chronically administered subcutaneously (sc) in male rats. Plasma T levels, and weights of the prostate, seminal vesicles (SV), and testes were evaluated. These effects were compared with those of leuprolide acetate (LA), a GnRH analogue, or bilateral orchiectomy (ORX). As mechanism of action studies, we assessed the pituitary responsiveness to LA by evaluating luteinizing hormone (LH) release, and the responsiveness of GnRH neurons to TAK-683 by evaluating c-fos immunoreactivity (ir), a marker of neuronal activity. We also determined GnRH peptide contents in the hypothalamus.
Results: TAK-448 and TAK-683 both were observed to be potent receptor agonists *in vitro*. Chronic administration of TAK-448 ($\geqslant$10 pmol/h) or TAK-683 ($\geqslant$30 pmol/h) reduced T levels to undetectable levels (<0.04 ng/mL) within 3−7 days, while LA (300 pmol/h) produced slower and less dramatic T reduction than metastin analogues. The weights of the prostate and SV were reduced by ~90% by metastin analogues or ORX, or by ~70% (prostate) and 80% (SV) by LA vs controls. The pituitary maintained LA responsiveness. Surprisingly, the GnRH neurons also maintained responsiveness via GPR54 since ~90% of GnRH neurons expressed c-fos-ir in response to TAK-683. However, hypothalamic GnRH contents were reduced after chronic TAK-683 administration.
Conclusions: TAK-448 and TAK-683 showed more efficient T suppression compared with LA in male rats, and TAK-448 possessed superior *in vivo* activity vs TAK-683. Chronic administration of metastin analogues appears to deplete GnRH from the hypothalamus, resulting in potent T suppression. Consequently, these compounds hold promise as therapeutic agents in hormone-related disorders, and their potential utilities in prostate cancer therapy will be discussed.

252 Withdrawn

253 Autophagy triggered by ursolic acid synergistically enhances 5-fluorouracil induced cell death in HCT15 (MSI p53 mutant) colorectal cancer cells

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Colorectal carcinoma (CRC) is a common cause of cancer-related death and tumours with microsatellite instability (MSI) and p53 mutations have been shown to be resistant to chemotherapy with 5-fluorouracil (5-FU). Therefore, it is essential to find compounds that could contribute to treatment efficacy by increasing the sensitivity to 5-FU. HCT15, a MSI human CRC derived cell line that harbours a p53 mutation, was incubated with the triterpenoid ursolic acid (UA) at a concentration that induces approximately 50% cell death (measured by PI stainning) after 48 h. A synergistic enhancement of apoptosis was observed when co-incubating 5-FU with UA (measured by TUNEL assay). UA induction of apoptosis was totally abolished by the JNK inhibitor SP600125 (SP), but not by the caspase inhibitor zVAD-fmk. Apoptosis did not account for all the observed cell death induced by UA. Thus, we asked whether UA was also inducing autophagy. We observed that UA induced