cured). BT 99-25 was stable in biological milieu with a half-life of approximately 72 hours. These results demonstrate that a phosphodiester 6-base length oligonucleotide, Oligomodulator™ BT 99-25, may have potential for the treatment of lymphoma.

566

Rhythms in BCL-2, cell cycle distribution and circadian clock gene expression in normal tissues and in tumor for improving novel targeted cancer therapy

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Objectives: To establish prerequisites for circadian timing of novel therapies targeted at cell cycle or apoptosis.
Methods: Two studies involved mice from 2 different strains (B6D2F1 and C3H/HeN, with or without mammary carcinoma) in order to assess 24-hour changes in cell cycle phase distribution (flow cytometry), clock gene mRNA (Per2, Bmal-1, Clock, Tim with RNAse protection) and BCL-2 protein (western blot) in bone marrow. The human oral mucosa was used to study (8 subjects), cell cycle proteins (immunohistochemistry), clock genes (hPer1, hBmal1, hClock, hTim, hCry1) and hBcl-2 (RT-PCR). 24-h changes were validated with ANOVA and cosinor. In the experimental models, a circadian rhythm was found for the proportion of G1 phase and S phase cells ($p < 0.05$) in the total bone marrow of both strains. BCL-2 expression varied 3 to 5-fold along the 24 h ($p < 0.02$), with a peak near the middle of the rest-phase of the rest-activity cycle. The transcriptional activity of mPer2 and mBmal1 varied rhythmically in mouse bone marrow and could control the BCL-2 rhythm. In mammary carcinoma-bearing C3H/HeN mice, the bone marrow cell cycle phase and BCL-2 rhythms were near normal. Circadian changes characterized G1 and G2/M phase in tumor cells ($p<0.01$), with peaks respectively occurring 2 and 7 h earlier than in bone marrow. No BCL-2 rhythm was found. The circadian regulation of cell cycle and apoptotic pathways was severely altered in this tumor known to display a marked circadian dependency of docetaxel efficacy (Granda et al. Cancer Res 2001). In humans, a 2- to 3-fold change along the 24-h time scale was documented for p53, and cyclins-E, -A and -B1, with peaks occurring at 11:00, 15:00, 16:00 and 21:00 respectively (Bjarnason et al. Am J Pathol 1999). These rhythms could result from the rhythmic expression of oral mucosa clock genes (Bjarnason et al. Am J 2001). Mucosal hBcl2 mRNA expression also varied by 50%, with a peak near 01:00 at night. Cell cycle and Bcl-2 pathways are under circadian clock regulation both in mouse bone marrow and in human oral mucosa, with a fixed relation with the rest-activity circadian cycle. In both species, BCL-2-mediated protection of normal cells against apoptotic processes may be increased during the rest span. These data support the incorporation of circadian concepts in the development of novel therapeutic agents targeting cell cycle and apoptosis. Supported in part by ARTBC, Villejuif and Aventis, Vitry, France

567

Cyclic AMP inhibits caspase-8-mediated, pH-dependent, apoptosis by attenuating cellular acidification

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The G protein-coupled receptor agonist somatostatin (SST) regulates multiple second messenger systems to inhibit cellular secretion and proliferation. Inhibition of secretion by SST is due, largely, to its ability to inhibit the activation of adenylylcyclase thereby decreasing cyclic AMP (cAMP) production. By contrast, the inhibition of cell proliferation by SST results from its positive regulation of the protein tyrosine phosphatase SHP-1. We have previously shown that activation of the cysteine aspartate-specific protease caspase-8 by SST promotes cytoplasmic acidification thereby triggering apoptosis in MCF-7 human breast cancer cells. In some models caspase-8-mediated apoptotic signalling occurs in the absence of any change in pHi. cAMP increasing agents have been shown to modulate apoptosis both positively and negatively. For instance cAMP can promote apoptosis either directly or by potentiating the action of a variety of apoptotic inducers in thymocytes and lymphocytes. By contrast it inhibits apoptosis in T lymphocytes, T cell hybridomas and in promonocytic leukemia cells. Likewise activation of adenylylcyclase directly by forskolin (Fsk) or by agonists of receptors that are positively coupled to this enzyme has been reported to either facilitate or suppress apoptosis. Whether these findings reflect differing effects of cAMP on pH-dependent and -independent apoptosis is not known. These considerations prompted us to investigate the effect of increased cAMP on SST- and TNF-a-induced apoptosis in MCF-7 cells. We report here that SST-, but not TNF-a-, induced apoptosis is acidification-dependent and is inhibited by elevated cAMP levels. The protective action of cAMP against SST-induced apoptosis is due both to an elevation of resting pHi and attenuation of SST-induced acidification distal to SHP-1-mediated caspase-8 activation. Interestingly, cAMP is not able to rescue the cells from SST-induced apoptosis once the pHi has fallen below 7.0. The cAMP-dependent protein kinase (PKA) inhibitor H-89 partially reversed the pH lowering effect of SST suggesting that both PKA-dependent and independent mechanisms mediate the protective action of cAMP on acidification. These findings reveal for the first time that cAMP can protect against SST-induced, acidification-dependent, apoptosis by attenuating the reduction in pHi.

568

Apoptosis signaling by 2-methoxyestradiol in DS-sarcoma cells

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Introduction: The anti-cancer potential of the natural estrogen metabolite 2-methoxyestradiol (2-ME) is independent of estrogen-receptor binding. Its selective growth inhibition of cancer cells and tumours is associated with microtubuli interaction, anti-angiogenetic effects and inhibition of superoxide dismutase. Recently, Huang et al. showed that 2-ME induces apoptosis in leukeamia cells by blocking the activity of superoxide dismutase which results in excessive production of superoxide anions. Addition of antioxidants or overexpression of superoxide dismutase prevents apoptotic cell death of these cells. In order to clarify whether this mechanism is generally induced by 2-ME in any cancer cells, the present study investigated apoptosis signaling of 2-ME in DS-sarcoma cells.
Results: Translocation of the pro-apoptotic protein Bax to the mitochondria was identified as initial apoptotic event, followed by a decrease in mitochondrial transmembrane potential and the release of AIF out of the mitochondria. In addition, upregulation of FasL and TNFa by 2-ME, two death receptor ligands, was observed. Although, 2-ME administration resulted in activation of caspases, pan caspase inhibitor Z-VAD-FMK could not block 2-ME induced apoptotic cell death pointing to a caspase-independent mechanism. Furthermore, an increase in formation of reactive oxygen species was observed after 2-ME treatment. However, supplementation with different antioxidants could not decrease the toxic effect of 2-ME.
Conclusion: These findings may indicate, that reactive oxygen species are not involved in apoptosis induction in DS-sarcoma cells, rather they are a consequence of mitochondrial damage. Thus we could not validate the results of Huang et al., who investigated the effect of 2-ME in leukaemia cells. Hence, the mechanism of apoptosis induction by 2-ME seems to be cell line dependent.

569

Modulation of STAT activation by DNA damaging anti-cancer drugs

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Members of the STAT (Signal Transducers and Activators of Transcription) family of transcription factors in particular STAT1, 3 and 5, have been shown to both promote and inhibit apoptosis. Normally, active STAT1 plays a role in mediating growth arrest and apoptosis in response to stimuli such as IFNg. It follows that its absence *in vivo* can lead to an increase in tumour formation. Furthermore, a number of tumour cell types show a defective response to STAT1 activation, accounting for a lack of growth arrest or apoptotic induction. Previously, we have shown that DNA-damaging anti-cancer drugs can activate NFkB, a transcription factor known to modulate apoptosis (1). We now show that the same family of drugs, which include the topoisomerase II-targeted drugs, doxorubucin and mitoxantrone, can also effect the duration and intensity of STAT1 activation. Specifically, we observed that treatment of the breast cancer cell line, MDA435, with IFNg results in activation of STAT1 as measured by increased phosphorylation of tyrosine 701. This activation was potentiated by both mitoxantrone and doxorubucin, drugs that give rise to DNA double stranded breaks. This potentiation was accompanied by enhanced nuclear localisation of STAT1 and modulation of downstream STAT1 targets. In addition, we observed a potentiation of