replication without altering the passage of the replication fork through telomeric DNA. Overall, these data are in agreement with the possibility that RHPS4 triggers a replication stress at telomeres, leading to an ATR-dependent DNA damage response.

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Biological activities of newly synthesized polyamine derivatives as potential anticancer agents

D. Baiocchi[1], L. Roncuzzi[2], E. Gilli[1], A. Milelli[3], V. Tumiatti[3], A. Gasperi-Campani[4]
[1]University of Bologna, Experimental Pathology, Bologna, Italy; [2] Istituti Ortopedici Rizzoli, Laboratory for Pathophysiology of Orthopedic Implants, Bologna, Italy; [3] University of Bologna, Pharmaceutical Sciences, Bologna, Italy [4] University of Bologna, Experimental Pathology and Interdepartmental Center for the Research on Cancer, Bologna, Italy

Natural polyamines are nitrogen-bearing aliphatic chains that play an essential role in cell growth and differentiation and represent physiological sources of polycations necessary for stabilization of DNA topologies. Polyamine content in the cells is tightly regulated: in addition to synthesis, mammalian cells are equipped with an efficient polyamine uptake system, whose activity is proportional to cell proliferation.

Polyamines analogues and derivatives can suppress proliferation of cancer cells by inhibition of the biosynthesis of natural polyamines and can exert cytotoxic activity due to their DNA-binding properties. Many tumour types have been shown to contain elevated level of an active polyamine transporter (PAT) for importing exogenous polyamines.

In the present study, a bis(benzyl)polyamine analogue (MDL 27695), known to exert antiproliferative activity, has been used as a template where to inserte a well-known DNA-intercalator group (aromatic core) to combine the ability to use the polyamine uptake system with the property to intercale and bind tightly the double-stranded DNA. For this purpose two groups of polyamine derivatives (symmetric or non-symmetric) were synthesized: each group includes 9 compounds which differ for the number of C atom (n=2-10) in the lateral chain to verify the best lenght of the amino-alkyl chain. All derivatives were tested for antiproliferative activity in human breast cancer (SKBR-3) and leukemia (CEM) in vitro in a range of concentrations beetween 0.1 and 10 μM. The MTT assay was used to determine growth inhibition after up 72 h of treatment.

Results indicate that the symmetric derivatives are more effective than the corresponding non-symmetric ones in both cell lines. All the symmetric compounds cause a significant dose- and time-dependent growth inhibition in the range of concentration tested. The compound with n=3 emerges as the most potent among the symmetric derivatives, with an IC_{50} (72h) of 0.35 and 0.17 μM in leukemic and breast cancer cells respectively.

The present preliminary results document that some of these polyamine derivatives are more active than polyamine DNA-Intercalator conjugates against human leukemia or than oxa-polyamines derivatives against breast cancer. The pharmacokinetics and pharmacodynamics of the active compounds are now under study.

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POSTER SESSION

Signalling pathways 2

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Combination of cetuximab with cisplatin and radiation therapy may have useful applications for the treatment of cervical cancer

D.D. Meira[1], V.H. Almeida[1], J.S. Mororó[1], I. Nóbrega[1], D.V.S. Batista[1], L.H. Bardella[1], R.L. Silva[1], R.M. Albano[2], C.G. Ferreira[1]
[1]Instituto Nacional de Câncer, Clinical Research, Rio de Janeiro, Brazil; [2] Universidade do Estado do RJ, Biochemistry, Rio de Janeiro, Brazil

Background: Cervical cancer kills 288,000 women each year, therefore, new therapeutic strategies are necessary. Here we examine the effects of cetuximab alone or in combination with cisplatin and radiation therapy (RxT) on cell cycle and proliferation, VEGF expression and antibody-dependent cellular cytotoxicity (ADCC) on the carcinoma cell lines A431 (vulva), Caski and C33A (cervical) and how these treatments influence downstream signaling pathways.

Methods: Cell lines were treated with cetuximab alone or in combination with CDDP or RxT, or with both, at different conditions. Cytotoxicity was assessed by clonogenic assays (CA) and cell cycle analysis by flow cytometry (FACS) using PI staining. Western blotting (WB) analysis was performed with antibodies against: EGFR (Tyr 845, 992, 1045 and 1068), AKT, HER2 and MAPK (total and phosphorylated). In vitro ADCC assay was done using the Cyto Tox 96® kit. VEGF expression was determined by real time RT-PCR and ELISA.

Results: A431 cells express 10^6 EGF receptors on the membrane while Caski and C33A cells express 20% and 1% of that, respectively (WB). Combination of cetuximab with RxT or cisplatin led to a stronger inhibition (n=3, $P<0.05$) of cell survival of A431, Caski and C33A cell lines than each treatment alone. Indeed, we observed at least an additive effect following the addition of cetuximab to RxT and cisplatin ($P<0.05$) due to an arrest at the G0/G1 phase of the cell cycle (FACS analysis) for all three cell lines tested. We performed WB to analyze the effects of cetuximab on phosphorylated EGFR, HER2, MAPK and AKT. Cetuximab could decrease the phosphorylation status of almost all residues in A431 and Caski cell lines. In the C33A cell line, which has low EGFR but high HER2 expression, cetuximab inhibits HER2 and MAPK phosphorylation, suggesting it has a large dependency on these pathways. Two factors leading to in vivo antitumor activity of anti-EGFR antibodies are the induction of ADCC and inhibition of angiogenesis. Cetuximab induced ADCC in 26.40% (A431), 15.1% (Caski) and 1.75% (C33A) of the cells at effector/target ratio of 20:1. Furthermore, cetuximab inhibited VEGF expression in all three cell lines.

Conclusions: We observed that cetuximab treatment plus cisplatin/RxT decreased A431, Caski and C33A cell survival, inhibited VEGF expression and induced ADCC. Our data suggest that cetuximab combined with cisplatin and RxT has useful applications for the treatment of cervical cancer.

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Celecoxib-induced apoptosis involves signaling through the Mcl-1/Noxa axis and can be blocked by overexpression of Bxl-xL but not Bcl-2

J. Rudner[1], S. Elsässer[1], A.C. Müller[1], C. Belka[1], V. Jendrossek[1]
[1]University of Tübingen, Department of Radiation Oncology, Tübingen, Germany

Introduction: The non-steroidal anti-inflammatory drug Celecoxib is known as a specific inhibitor of Cyclooxygenase-2 (Cox-2). Cox-2 has been shown to be overexpressed in many tumors and can contribute to an enhanced resistance towards apoptosis induction by certain apoptotic stimuli. Recent experiments in Cox-2 negative cell lines, however, suggest that Celecoxib is able to induce apoptosis in a Cox-2-independent manner, probably through the mitochondrial pathway. Mitochondrial integrity is controlled by the pro- and anti-apoptotic members of the Bcl-2 family. To date, however, it remains unclear which specific members are needed to be activated and neutralized for a successful induction of apoptosis, especially in response to Celecoxib.

Methods: Regulation of Celecoxib-induced apoptosis was analyzed in Jurkat T cell lymphoma cells which were transfected with Bcl-2, Bcl-xL, or the empty vector. The implication of Mcl-1 and Noxa was determined by transfecting the cells with the respective siRNA. Induction of apoptosis was determined by flow cytometry and fluorescence microscopy. Downregulation of protein expression and caspase activation during apoptosis was verified by Western blot analysis. cytochrome c release was assayed by fluorescence microscopy and cellular fractionation with subsequent Western blot analysis.

Results: Celecoxib induced apoptosis in the Cox-2 negative Jurkat T-cell lymphoma cell line through the mitochondrial pathway that involves the breakdown of the $\Delta\psi m$. During Celecoxib-induced apoptosis the pro-apoptotic protein Bak was activated and cytochrome c was released into the cytosol.

Examination of the members of the Bcl-2 family revealed that the anti-apoptotic Mcl-1 was down-regulated whereas the expression level of the pro-apoptotic BH3-only protein Noxa remained unchanged. Mcl-1 co-immunoprecipitated with Noxa. However, this interaction was diminished 6h after treatment with Celecoxib due to the downregulation of Mcl-1.

Mcl-1 downregulation by siRNA was sufficient to induce apoptosis within 3 hours in Jurkat T cells. Although the downregulation of Noxa by siRNA did not induce apoptosis it made the cells more resistant towards Celecoxib-induced apotosis.

The induction of apoptosis by Celecoxib could be inhibited by overexpression of the anti-apoptotic Bcl-xL but not by the closely related Bcl-2 although Mcl-1 downregulation was not inhibited by these two anti-apoptotic proteins.

Conclusions: Celecoxib induced apoptosis through the Mcl-1/Noxa axis in Jurkat T-cells. An early step in the induction of apoptosis by Celecoxib was the downregulation of Mcl-1. Despite the neutralization of Mcl-1, apoptosis induction could be blocked by overexpression Bcl-xL but not Bcl-2 implying a different role of Bcl-2 and Bcl-xL in Celecoxib-induced apoptosis.