no difference in proliferation and cell death characteristics in proliferating, adherent monolayer cell cultures of CLDN1-positive compared to control CLDN1-negative and mock-transduced cell cultures. MDA-MB 361 parental cells exhibited no changes in cell death induction either in 2D monolayer or in 3D spheroid cell cultures. In contrast, clonal CLDN1-transduced derivatives displayed a significant elevation of apoptosis which became evident as early as 2 days after 3D spheroid culture onset. The clonal MDA-MB 361 CLDN1-positive cultures which exhibited a more prominent cell membrane localization showed a pronounced increase of apoptosis in tumor spheroids up to 8-fold over the CLDN1-negative control. In parallel, inhibition of the paracellular flux rate was observed. These findings support the potential role of the TJ protein CLDN1 in breast cancer cells to restrict nutrient and growth factor supplies, and indicate that the loss of the cell membrane localization of the TJ protein CLDN1 in carcinomas is likely a crucial step during the process of tumorigenesis.

546

DNA damage induces a novel p53-survivin signaling pathway regulating cell cycle and apoptosis in acute lymphoblastic leukemia cells

M. Zhou[1], L. Gu[1], F. Li[2], Y. Zhu[1], W. Woods[1], H. Findley[1]. [1]*Emory University, Pediatics, Atlanta, USA;* [2]*Roswell Park Cancer Institute, Pharmacology and Therapeutics, Buffalo, USA*

Survivin is a novel member of the inhibitor of apoptosis protein (IAP) family. Here we report that the chemotherapeutic drug doxorubicin, a DNA-damaging agent, activates a p53-survivin signaling pathway inducing cell cycle arrest and apoptosis in childhood acute lymphoblastic leukemia (ALL). Treatment of wild-type (wt) p53 ALL cells (EU-3 cell line) with doxorubicin caused accumulation of p53, resulting in dramatic downregulation of survivin, depletion of cells in G2/M and apoptosis (increased sub-G1 compartment). In contrast, doxorubicin treatment of mutant (mut) p53 cells (EU-6/ALL line) upregulated survivin and induced G2/M arrest without inducing apoptosis. However, treating EU-6 with anti-survivin antisense re-sensitized these cells to doxorubicin, resulting in apoptosis. With a p53-null cell line (EU-4), although doxorubicin treatment arrested cells in G2/M, survivin expression was unchanged, and cells underwent only limited apoptosis. However, re-expression of wt-p53 in EU-4 cells could restore the doxorubicin-p53-survivin pathway, resulting in significantly decreased survivin expression and increased apoptosis in these cells after doxorubicin treatment. Following cotransfection of p53-null EU-4 cells with survivin promoter-luciferase constructs and either wt-p53 or different mut-p53 expression vectors, wt-p53 inhibited survivin promoter activity; p53-mediated inhibition could be abrogated by overexpression of MDM2 protein. Together, these studies define a novel p53-survivin signaling pathway activated by DNA damage that results in downregulation of survivin, cell-cycle arrest and apoptosis. Furthermore, our data indicate that loss of wt-p53 function in tumor cells may contribute to upregulation of survivin and resistance to DNA-damaging agents.

547

Small molecule inhibitors of BCL-2 protein-protein interactions show anti-tumor activity in nude mice

P. Beauparlant[1], G. Attardo[1], L. Belec[1], R. Marcellus[1], H. Chan[1], A. Jang[1], N.A.E. Steenaart[1], M.S.R. Murthy[1], M. Jamali[2], G.C. Shore[1]. [1]*Gemin X Biotechnologies Inc., Montreal, Canada;* [2]*Lady Davis Institute, Montreal, Canada*

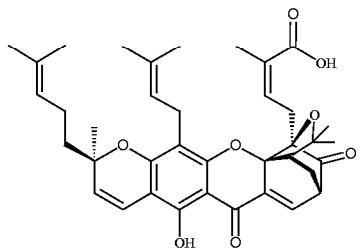
Oncogenes are potent activators of the apoptotic response and cells must abrogate the cell death response in order to survive and form tumors. Many cancers achieve this through upregulation of the BCL-2 family dominant suppressors of apoptosis. These proteins function through protein-protein interactions to ablate the oncogene-induced pro-apoptotic BAX/BAK mediated release of signals from mitochondria, resulting in activation of caspases. As previously shown, the prodigiosin GX01 compound series (Mr < 400) inhibit BCL-2/BAX interactions, and while these compounds readily enter both normal and cancer cells, the cancer cells show a significantly greater apoptotic response at low nM concentrations. Here we show that when Jurkat cells were treated with 100 nM GX015-000, loss of cell viability as measured by ViaLight ATP detection was seen over a period of 72 hours. Consistent with a BCL-2 inhibition, cell cycle analysis by flow cytometry did not reveal any major accumulation at a distinct cell cycle phase, suggesting that GX015 does not cause a cell cycle arrest. At the 72 hour point, there were a large number of apoptotic sub-diploid cells and degraded cell debris was apparent. To determine the anti-cancer activity of GX015 compounds, murine xenograft tumor models were used. Nude mice (15 mice per group) were injected subcutaneously with C33-A human cervical carcinoma cells or human prostatic PC3 carcinoma cells, both of which over-express BCL-2 family members. Once the tumors became palpable, an oil emulsion of GX015-000 was administered subcutaneously at a site distal from the tumor. The mice tolerated doses up to 10 mg/kg per day and doses equal to 3.4 mg/kg per day or greater inhibited tumor growth. The relative hydrophobicity of GX015-000 (log P = 6.4) suggested that adequate tumor cell coverage in xenograft models might be difficult to achieve for optimal tumor regression. Accordingly, GX015-000 was found to be 99% bound to plasma protein. To address this issue, less lipophilic GX015 compounds were identified. GX015-003 has a Log P of 3.2 and only 1% of this compound is bound to plasma proteins. GX015-003 can effectively (IC_{50} in C33A cells of 40 nM) and selectively induce apoptosis in cancer cells as measured by caspase activation. GX015-003 is being tested for anti-cancer activity in nude mice bearing C33A subcutaneous tumors. The pharmacokinetic profile of GX015-003 in comparison to GX015-000 is also being determined.

548

Discovery of gambogic acid and derivatives as apoptosis-inducing natural products with novel mechanism of action and potent *in vivo* anti-tumor activity

S. Kasibhatla[1], L. Francis[2], S. Maliartchouk[3], J. Morrow[4], H.-Z. Zhang[5], K. Ollis-Mason[6], J. Drewe[7], S.X. Cai[8], B. Tseng[9]. [1]*Maxim Pharmaceuticals, Inc., Biology;* [2]*Maxim Pharmaceuticals, Inc., Biology;* [3]*Maxim Pharmaceuticals, Inc., Biology;* [4]*Maxim Pharmaceuticals, Inc., Biology;* [5]*Maxim Pharmaceuticals, Inc., Chemistry;* [6]*Maxim Pharmaceuticals, Inc., Chemistry;* [7]*Maxim Pharmaceuticals, Inc., Biology;* [8]*Maxim Pharmaceuticals, Inc., Chemistry;* [10]*Maxim Pharmaceuticals, Inc., Biology, San Diego, USA*

Apoptosis is a physiological form of cell death controlled by a family of cysteine proteases called caspases. Aberrant apoptosis is not only the major cause for tumor development and progression, but also plays a significant role in drug resistance to conventional treatments. Therefore, discovery of novel inducers of apoptosis provides a new therapeutic approach to anti-cancer agents. Here we report the discovery of gambogic acid (MX2060), a natural product isolated from gamboge, as a novel inducer of apoptosis in different cancer cell lines, including breast, prostate, and other cell lines. Treated cell lines also exhibited a lack of clonogenic survival. Caspase activation (EC50), ranges from 0.5 μM to 1.5 μM for different cell lines. The signaling pathway includes activation of the upstream caspase 8, as well as involvement of mitochondria and release of cytochrome c, thus engaging both the intrinsic and extrinsic pathways of cell death. Mitochondria play an important role in the regulation of apoptosis and an early event in this pathway is the release of apoptogenic cytochrome c from the mitochondria into the cytosol that is inhibited by the anti-apoptotic Bcl2 family members. MX2060-induced cell death is delayed, but not inhibited by, Bcl2 suggesting its effectiveness in tumors overexpressing Bcl2. Cells treated with MX2060 undergo apoptosis with cleavage of all key caspase substrates and rapid cell death independent of the cell cycle stage, which may offer an advantage over some of the current chemotherapeutic drugs. MX2060 also exhibited a pharmaceutically acceptable pharmacokinetic profile following i.v. administration. In order to understand the Structure Activity Relationship (SAR) and improve the chemical and pharmacological properties of MX2060, we have designed and synthesized many derivatives. We have found that MX2060 and its derivatives have good *in vivo* efficacy in mouse xenograft models. We will report the key SAR, *in vitro* and *in vivo* characterization of MX2060 and its derivatives.



In summary, MX2060 represents a new chemotype whose apoptosis-inducing antitumor activity appears to be mediated by a molecular mechanism different from the action of traditional cancer drugs. The therapeutic implications of MX2060 will be discussed.