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Nuclear factor-kB activation. a new target for drug design?

W. Priebe[1], K. Ashikawa[1], I. Fokt[1], T. Inge[2], B.B. Aggarwal[1]. [1]*The University of Texas M. D. Anderson Cancer Cente, Bioimmunotherapy, Houston;* [2]*University of Cincinnati, Children's Hospital and Medical Center, Division of Pediatric Surgery, Houston, USA*

Activation of nuclear factor (NF)-kappaB is thought to suppress apoptosis through expression of antiapoptotic genes; however, numerous studies have documented that clinically effective, highly apoptotic antitumor agents like camptothecin, paclitaxel, vinblastin, vincristine, fluorouracil, methotrexate, and doxorubicin (DOX) all activate NF-kB. We studied whether DOX, WP744, a structural analog of DOX with high proapoptotic properties and increased cytotoxicity, and WP631, a daunorubicin related novel bisintercalating agent activate NF-kB and whether this activation is essential for apoptosis in myeloid (KBM5) and lymphoid (Jurkat) cells. We found that all tested compounds had antiproliferative effects against KBM5 cells and that WP744 was the most potent, with an IC_{50} of 0.5 mM; DOX was the least active, with an IC_{50} of 2 mM, as determined by Trypan blue exclusion and by thymidine incorporation.NF-kB activation examined by electrophoretic mobility gel shift assay revealed that NF-kB activation was maximal at 1 mM for WP744 and at 50 mM for DOX and WP631. NF-kB activation was associated with IkBa degradation; time course studies showed that NF-kB activation and IkBa degradation preceded the cytotoxic effects of DOX. The participation of NF-kB in drug-induced cytotoxicity was confirmed using two methods. First, lymphoid (Jurkat) cells, which are deficient in the receptor-interacting protein (RIP) needed for NF-kB activation were examined. All three anthracyclines activated NF-kB in control Jurkat cells but none did in RIP-deficient Jurkat cells; moreover, the control cells were sensitive to all 3 compounds but the RIP-deficient Jurkat cells were completely resistant. Secondly, the NF-kB inhibitor pyrrolidine dithiocarbamate profoundly protected against apoptosis and death of SK-N-SH neuroblastoma cells in response to WP744. Thus, two lines of evidence confirm that NF-kB activation is essential for apoptosis induced by novel anthracyclines. In summary: NF-kB activation and IkBa degradation seem to be early events activated by DOX and its analogs that play a critical pro-apoptotic role. NF-kB activation also is strongly affected by relatively small structural modifications, as exemplified by WP744, suggesting that NF-kB activation might be a marker of the effectiveness of specific modifications of DOX and other important apoptotic antitumor agents.

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A novel target for antifolates: the dihydrofolate reductase domain of the G1/S transit controlling protein eIF-5A

H.M. Hanauske-Abel[1], A.-R. Hanauske[2], B.M. Cracchiolo[3], E. Wolff[4], M.H. Park[4], W. Gordon[5], A.M. Popowicz[5]. [1]*New Jersey Medical School - UMDNJ, Pediatrics and Obstetrics, Gynecology;* [2]*AK St. Georg, Medical Oncology, Hamburg, Germany;* [3]*New Jersey Medical School - UMDNJ, Obstetrics, Gynecology;* [4]*National Institutes of Health, National Institute of Dental and Craniofacial Resea, Bethesda;* [5]*Rockefeller University, Computing Services, New York, USA*

The two isoforms of the eukaryotic translation initiation factor 5A (eIF-5A) each contains a single hypusine residue, formed by deoxyhypusyl hydroxylase (DOHH) within a collagen motif. DOHH inhibitors (BBA 1077, 159; 1991) cause cell cycle arrest at the immediate G1/S boundary (BBA 1221, 115; 1994) jointly with the disappearance from polysomes of a unique subset of cellular mRNAs (FEBS Lett 366, 92; 1995) termed 'hymns'. Upon DOHH reactivation, hymns reappear at polysomes, followed by synchronized entry of cells into S phase. Though encoding diverse cell cycle-relevant proteins, hymns display joint motifs (JSBs) in their 3'- and 5'-UTRs. Recent analyses indicate that the C-terminal part of eIF-5A folds like the cold-shock protein A of E.coli, which prevents mRNA duplexes at low temperature (Structure 6, 1207; 1998), and that the N-terminal part contains five motifs of ATP-utilizing mRNA helicases, required for unwinding of mRNA duplexes (FASEB J 16, A549; 2002). Hypothesizing that eIF-5A databases contain further clues for direct interaction with specific mRNAs, we refined search parameters and strategies. Using the spatial coordinates of the N-terminal part of eIF-5A of M. jannaschii (PDB# 1EIF), we noted homology with the crystal structure of dihydrofolate reductase (DHFR) of E. coli (PDB# 1vie; Dali algorithm Z score = 4.4). Optimized alignment between human DHFR (XM_165390) and the human eIF-5As (I: NP_001961; II: NP_065123) revealed 37% identify/similarity with eIF-5A-I, and 35% with eIF-5A-II. The human proteins share isolocated residues that in DHFR participate in binding of folate and methotrexate (e.g. Ile7, Pro61, Arg70) and of NADPH (e.g. Gly20, Lys54, Ser118), though distinct differences, e.g. the E30Q isolocation, suggest DHFR activity of eIF5As is limited. However, human DHFR not only serves as a catalyst, it also interacts with and masks its cognate mRNA, decreasing translation; methotrexate inhibits mRNA binding by DHFR (Biochemistry 36, 12317; 1997). We propose that with regard to hymns, the DHFR domain of eIF-5 also functions in translational control. We postulate that antifolates like methotrexate or Alimta (LY23154) block this function, rendering hymns untranslatable akin to DOHH inhibition. Of note, like DOHH antagonists, both antifolates can inhibit G1/S transit (Exp Cell Res 170, 93;1987; Anticancer Res 21, 3209; 2001). These findings reinforce our original proposal that eIF-5A is a target for chemotherapy (Blut 59, 286; 1989).

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Targeting Jak3 with small molecules to inhibit T-cell activity

W. Priebe[1], Z. Nagy[2], S.M. Stepkowski[3], S. Kosinski[1], I. Fokt[1], B.D. Kahan[3], R.A. Kirken[2]. [1]*The University of Texas M.D. Anderson Cancer Center, Dept. of Bioimmunotherapy, Houston;* [2]*The University of Texas Houston Health Science Center, Dept. of Integrative Biology, Houston;* [3]*The University of Texas Houston Health Science Center, Dept. of Surgery, Houston, USA*

Janus kinase 3 (Jak3) is a cytoplasmic tyrosine kinase associated with the interleukin (IL) -2 receptor common gamma chain and is activated by multiple T-cell growth factors such as IL-2, -4, and -7. Mice and humans deficient in Jak3 show severely impaired immune response. Unlike the three other Jak family members, Jak3 is confined to lymphocytes, monocytes, and natural killer (NK) cells, making it an attractive therapeutic target for T-cell-mediated diseases. Using human T cells, we documented that small molecules belonging to the tyrphostin family, undecylprodigiosin congeners, and other new small molecules can block, with various degrees of selectivity, T-cell responses, including cytokine-mediated cell growth. We also identified agents showing selectivity for Jak3 as measured by autokinase assays and activation of downstream substrates including the signal transducers and activators of transcription Stat5a and Stat5b and mitogen-activated protein kinase (Mapk) cascade effectors. Selectivity was demonstrated by our findings that growth of non-Jak3-expressing T cells was not affected by these drugs as compared with primary human T cells and Jak3-expressing cell lines responsive to IL-2. Selectivity for Jak3 as opposed to Jak2 was also demonstrated by showing within the same T-cell model (Nb2) that IL-2-Jak3-mediated signals were disrupted but prolactin-Jak2-mediated signals showed minimal effects as determined by cell growth and Jak2/Stat5 activation profiles. We are currently exploring these agents as potentially useful and selective agents against lymphoma and myeloma.

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N-3 fatty acids improve treatment efficacy of Phor14-beta3 in nude mice with prostate cancer xenografts

C. Leuschner[1], W. Hardman[2], F. Enright[3], W. Hansel[4]. [1]*Pennington Biomedical Research Center, Reproductive Biology, Baton Rouge, USA;* [2]*Pennington Biomedical Research Center, Cancer and Nutrition, Baton Rouge, USA;* [3]*Lousiana State University, Veterinary Science, Baton Rouge, USA;* [4]*Biomedical Research Center, Reproductive Biology, Baton Rouge, USA*

We prepared a conjugate of a lytic peptide (Phor14) and a 15 amino acid sequence of the β chain of chorionic gonadotropin (beta 3). Previous studies on treatment of PC-3 prostate cancer xenografts in nude mice showed that PC-3 tumors expressing the LH/CG receptors can be destroyed by lytic peptide LH/CG conjugates (Leuschner et al Prostate 46,116). In the current study we determined whether supplementing the diet with n-3 FAs would increase the efficacy of treatment with the lytic peptide conjugate. The diet was based on the AIN-76A diet and contained either 10 % corn oil (CO) or 8% corn oil + 2% n-3 FAs concentrate. The CO or the n-3 FAs containing diets were fed to PC-3 tumor bearing mice 10 days prior to treatment with Phor14-beta3. On day 21, 28, 35, 42 and 49 post tumor inoculation, both groups of mice were treated with 0 (saline) or 10 or 20 mg/kg Phor14-beta3 via tail vein injections. N-3 FAs fed mice had higher body weights and smoother skin texture during the entire study than CO fed mice indicating less tumor associated cachexia. Phor14-beta3 treatment reduced tumor weight in a dose dependent manner in the CO fed group [mean ± SD tumor weights: saline controls, 2.9 ± 1.4 g; 10 mg/kg Phor14-beta3, 1.8+0.8g ($p<0.1$ vs 20 mg/kg Phor14-beta3); 20 mg/kg Phor14-beta3, 1.3 + 0.6 g ($p<0.02$ vs saline control)]. However, in the n-3 FAs fed group, the reduction in tumor weight was not dose dependent [mean ± SD tumor burdens: saline control, 2.89+0.6 g ($p<0.02$ vs treatments); 10 mg/kg Phor14-beta3,