activated telomerease, as shown by immuno-detection of telomerase and TRAP assays. Furthermore, inhibition of BARD1 expression by siRNA transfection leads to loss of telomeric sequences, telomeric fusions, and chromosomal translocations in mouse mammary gland cells TAC-2, primary rat mesothelial cells, and Hela, or MCF-7 cells. This indicates that BARD1 controls telomere structures independently of telomerase activity. A fraction of BARD1 co-localizes with telomeres, as well as with the bona fide telomere binding protein TRF2, shown by FISH and immuno-staining and DNA chip assay. Our data are consistent with the following model: i) BARD1 might be sequestered on telomeres, ii) critically short telomeres would lead to an increase of unbound BARD1, iii) free BARD1 could act in apoptosis induction by binding and stabilizing p53 as demonstrated previously (Irminger-Finger Mol Cell 2001). Repression of BARD1 allows cells with critically short telomeres continue to divide. Due to genomic instability, eventually, some of these cells can activate telomerase and therefore pass crisis.

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Both RIP and c-FLIP are required for inhibition of caspase-8 cleavage in TRAIL-DISC in human cancer cells

J.H. Song[1], D.K. Song[1], N.M. Kneteman[2], C. Hao[1]. [1]*University of Alberta, Laboratory Medicine and Pathology, Edmonton, Canada;* [3]*University of Alberta, Surgery, Edmonton, Canada*

Background: Many cancer cells express the death receptors DR4 and DR5 for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) yet are resistant to TRAIL-induced apoptosis. TRAIL induces apoptosis through the recruitment of Fas-associated death domain (FADD) and caspase-8 to DR4/DR5, leading to assembly of death-inducing signaling complex (DISC) where caspase-8 is cleaved and initiates apoptosis. To explore the mechanisms in TRAIL resistance, we analyzed TRAIL-DISC in resistant cancer cells.
Methods: Non-small cell lung carcinoma A549, H596 and H1792, colon carcinoma Caco-2 and Colo320, breast carcinoma MB456, and pancreatic carcinoma Panc-1 cell lines were included in the study. TRAIL-DISC was immunoprecipitated using Flag-tagged TRAIL and anti-Flag M2 antibody and examined on Western blots. Cell death was analyzed by acid phosphatase assay and caspase cleavage was examined on Western blots. Synthetic small interfering RNA (siRNA) was generated by Qiagen Inc. and transfected with TransMessenger transfection reagent.
Results: Receptor-interacting protein (RIP) and cellular Fas-associate death domain-like, IL-1β-converting enzyme-inhibitory protein (c-FLIP) were reported in TRAIL-DISC. Western blot analysis of TRAIL-DISC revealed RIP and c-FLIP in TRAIL resistant A549 and H596, but not sensitive H1792 cells. Western blots detected cleavage products of caspase-8, caspase-3 and DNA fragmentation factor 45 (DFF45) in the cytoplasm in the sensitive, but not in the resistant cells. Transfection of siRNA targeting *c-FLIP* gene inhibits c-FLIP expression and sensitized the resistant cells to TRAIL-induced apoptosis through caspase-8-mediated caspase cascade. The results suggested that c-FLIP inhibited caspase-8 cleavage in the DISC in the resistant cells. Detection of RIP-mediated inhibitor of κB kinase (IKK) complex in the DISC suggested that RIP mediated IKK-mediated nuclear factor-κB (NF-κB) activation in resistant cells. Transfection of siRNA specific to *RIP* gene, however, sensitized the resistant cells to TRAIL-induced apoptosis through caspase-8-mediated caspase cascade. Transfection of either RIP or c-FLIP siRNA in TRAIL resistant Caco-2, Colo320, MB456 and Panc-1 cell lines resulted in the cell sensitivity to TRAIL-induced apoptosis.
Conclusion: The results indicate that both RIP and c-FLIP are required for inhibition of caspase-8 cleavage in TRAIL-DISC and thus targeting either RIP or c-FLIP may provide novel therapeutic strategies in cancer therapies.

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Mutant p53 reactivation by PRIMA-1: a novel strategy for cancer therapy

K. Wiman[1], V. Bykov[1], N. Issaeva[2], N. Zache[1], J. Bergman[3], G. Selivanova[2]. [1]*Karolinska Institute, Oncology-Pathology, Stockholm, Sweden;* [2]*Karolinska Institute, Microbiology & Tumor Biology Center, Stockholm, Sweden;* [3]*Karolinska Institute, Dept of Biosciences, Stockholm, Sweden*

Mutant p53 reactivation in tumors should trigger massive apoptosis and thus eliminate the tumor. We previously identified PRIMA-1, a low molecular weight compound that reactivates mutant p53 and induces robust apoptosis in human tumor cells. Intravenous adminstration of PRIMA-1 inhibited human tumor xenograft growth in mice (1). These results were corroborated by our statistical analysis of available information in the National Cancer Institute database (2). Growth inhibition profiles for PRIMA-1 and known anticancer agents were analyzed. PRIMA-1 was consistently more efficient in inhibiting growth of mutant p53-carrying tumor cell lines compared to wild type p53-carrying lines, and sensitivity to PRIMA-1 was correlated to mutant p53 expression levels. This distinguishes PRIMA-1 from most known anticancer drugs which preferentially affect tumor cells carrying wild type p53. We have found that PRIMA-1 acts synergistically with several anticancer drugs to inhibit tumor cell growth. Our further studies revealed that PRIMA-1 and cisplatin showed synergistic induction of apoptosis in a mutant p53-dependent manner. Combined systemic treatment with low doses of PRIMA-1 and cisplatin produced a significant synergistic antitumor effect in mice carrying human tumor xenografts. Enhancement of mutant p53 expression levels by DNA-damaging chemotherapeutic drugs may increase sensitivity to PRIMA-1-induced apoptosis. The anticancer efficacy of PRIMA-1 will be tested in clinical trials. Reactivation of mutant p53 by PRIMA-1 alone or in combination with conventional chemotherapeutic drugs is a novel strategy for cancer therapy that should allow efficient elimination of mutant p53-carrying tumors.

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Analysis of survivin splice variant transcripts in human breast tumor cells

S. Lizard-Nacol[1], F. Vegran[1], R. Boidot[1], C. Oudin[1], B. Coudert[2]. [1]*Centre GF Leclerc, Molecular Genetics, Dijon, France;* [2]*Centre GF Leclerc, Oncology, Dijon, France*

Background: Survivin, is a member of the apoptosis inhibitors family, and is expressed in several human tumors. Two alternative splice variants of survivin (survivin-DEx3, and survivin-2B) differing in their anti-apoptotic properties were recently identified. While the anti-apoptotic effect of survivin-DEx3 is preserved, survivin-2B has lost its anti-apoptotic potential and may act as a naturally occurring antagonist of survivin and survivin-DEx3. Because in vivo studies have reported absence of survivin-2B in some cases of tumor progression, we analyzed the expression of these transcripts in breast cancer.
Material and Methods: reverse transcriptase polymerase chain reaction was performed using RNA samples obtained from 2 groups of breast cancer: node-negative (N−) and locally advanced (LA) tumors.
Results: all survivin variants were expressed in a majority of tumor samples, with survivin variant being the most dominant. In contrast, survivin-2B expression frequency was higher in N− (37/40: 93%) than in LA tumors (56/84: 67%; p=0.002).
Conclusions: These results demonstrated the expression of survivin splice variants in breast tumors and strongly suggested that the absence of expression of survivin-2B could be related to tumor progression in this disease.

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The proteasome inhibitor bortezomib (VELCADE™) sensitizes human tumor cells to TRAIL-mediated apoptosis by reduction of c-FLIP

T.J. Sayers[1], A.D. Brooks[1], J. Onksen[2], P.J. Elliott[3], W.J. Murphy[4]. [1]*SAIC Frederick, NCI Frederick, Laboratory of Experimental Immunology, Frederick, USA;* [2]*NCI Frederick, Laboratory of Experimental Immunology, Frederick, USA;* [3]*Combinatorx, Product Development, Boston, USA;* [4]*University of Nevada, Reno, Department of Microbiology, Reno, USA*

We have previously reported that the proteasome inhibitor bortezomib (PS-341, VELCADE™) can sensitize a murine acute myeloid leukemia (C1498) and a murine renal cancer (Renca) to TRAIL-mediated apoptosis. The effects of bortezomid and TRAIL were selective, since this combination could purge C1498 tumor cells from bone marrow, without major effects on normal bone marrow cells. Surprisingly, sensitization of these murine tumor cells was independent of any effect of bortezomib on NF-kB activation, yet did correlate with a reduction in levels of the anti-apoptotic protein c-FLIP. Utilizing the NCI panel of 60 human tumor cell lines, we assessed the sensitivity of a wide variety of different human cancer cell lines to the combination of bortezomid and TRAIL. A significant number of the tumor cell lines (20–30%) were dramatically sensitized to TRAIL-mediated apoptosis by treatment with bortezomib (20nM). However, for the remainder of the tumor cell lines, no such sensitization occurred. No