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Identification and evaluation of PI3 Kinase as new molecular target in neuroblastoma that critically regulates apoptosis resistance

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While aberrant activation of the PI3 Kinase (PI3K)/Akt pathway, a key survival cascade, has previously been linked to poor prognosis in several human malignancies, the question whether PI3K/Akt presents a suitable target for therapeutic intervention in neuroblastoma has not yet been answered. Therefore, we evaluated the PI3K/Akt pathway as molecular target in neuroblastoma in the present study. Analysis of the phosphorylation status of key components of the PI3K/Akt cascade in 116 primary neuroblastoma samples by tissue microarray reveals that phosphorylation of Akt at serine 473 (S473) and/or threonine 308 (T308), S6 ribosomal protein and ERK frequently occurs in primary neuroblastoma. Importantly, Akt phosphorylation significantly correlates with decreased event-free or overall survival in neuroblastoma, whereas the phosphorylation status of S6 ribosomal protein or ERK has no prognostic impact. Of note, monitoring Akt at T308 or both phosphorylation sites improves the prognostic significance of Akt activation in neuroblastoma specimens compared to S473 phosphorylation. In addition, Akt activation correlates with several parameters of aggressive disease, including MYCN amplification, 1p36 aberrations, advanced disease stage, age at diagnosis and unfavorable histology. In parallel experiments in neuroblastoma cell lines, activation of Akt by IGF-1 significantly inhibits TRAIL- or chemotherapy-induced apoptosis in a PI3K-dependent manner. Accordingly, inhibition of IGF-1-mediated Akt activation by the PI3K inhibitor LY294002 also completely reverses the IGF-1-imposed protection of neuroblastoma cells from TRAIL- and anticancer drugs-triggered apoptosis. Furthermore, blockade of constitutive Akt activation by a small molecule class I PI3K inhibitor synergizes with death receptor ligands or various chemotherapeutic drugs to trigger apoptosis in neuroblastoma cells. Analysis of signaling pathways reveals that PI3K inhibitors profoundly enhance TRAIL-induced caspase activation, mitochondrial membrane permeabilization and DNA fragmentation, providing a molecular explaination for the synergistic antitumor activity. In conclusion, we identify Akt activation as a novel prognostic indicator of poor outcome in neuroblastoma. Importantly, small molecule PI3K inhibitors overcome apoptosis resistance and prime neuroblastoma cells for death receptor- and chemotherapy-induced cell death. Thus, PI3K presents a novel, clinically relevant molecular target in neuroblastoma that warrants further exploitation.

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Combination effects of SF1126, a vascular targeted PI3 kinase inhibitor, with herceptin in Her-2 overexpressing breast cancer cells

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Background: Overexpression of Her-2/neu, seen in approximately 30% of breast cancers, is associated with poor prognosis. Trastuzumab (Herceptin), a humanized monoclonal antibody to HER2, has limited effectiveness and patients who respond to trastuzumab will relapse despite continued treatment. Preclinical studies have indicated that activation of the phosphatidylinositol 3-kinase/Akt (PI3-Kinase) pathway could contribute to trastuzumab resistance.
SF1126, combines a peptide integrin-targeted linked to a small pan PI-3 kinase inhibitor, developed to increase solubility and delivery of the active compound to the tumor. Recent studies have shown that SF1126 has both antitumor and antiangiogenic activity in vivo. This study was designed to explore the ability of SF1126 to enhance Herceptin efficacy in Her2-overexpressing breast cancer cell line.
Materials and Methods: BT474 cells were treated with Herceptin (0.1–100 μg/ml) or SF1126 (3.75−90 μM). Combination treatments were assessed using Herceptin at increasing doses with a fixed dose of SF1126 (IC50: 25 μM) or increasing doses of SF1126 with IC50 Herceptin (2.5 μg/ml), using the MTT assay following 72 h drug exposure. For Western blot analysis, BT474 cells were treated for 30 min with SF1126 (25 & 50 μM) and Herceptin (2.5 μg/ml), as single agents or in combinations.
Results: Treatment with Herceptin and SF1126, as single agents, inhibited the growth of BT474 cells in a dose dependent manner. When various concentrations of Herceptin, or SF1126, were combined with a fixed dose of SF1126, or Herceptin, respectively, synergistic enhancement of Herceptin efficacy were obtained (CI < 1). Western blot analysis revealed that SF1126 downregulated p-Her2, p-AKT and the downstream p-mTOR, p-4EBP-1 & p-p70S6K. The combined treatment resulted in a further downregulation of this signaling pathway. The effects of SF1126 on angiogenesis and programmed cell death were also investigated. SF1126 caused an inhibition of hypoxia-driven HIF-1α stabilization/accumulation in these cells and an increase of cleaved caspase 3 in a dose- and time-dependent fashion.
Conclusions: Here we report that co-treatment with SF1126 enhanced Herceptin efficacy in BT474 cells and downregulated the Her-2 pathway, p-AKT and its downstream effectors. We further demonstrate that SF1126 controls the hypoxic-stabilization of HIF-1α and caspase 3-dependent apoptosis. In vivo xenograft studies are ongoing and will be presented at the meeting.

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PI3 Kinase inhibitors broadly sensitize glioblastoma cells for death receptor- or chemotherapy-induced apoptosis

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Activation of the PI3 Kinase (PI3K)/Akt/mTOR pathway has recently been reported to correlate with increasing tumor grade, decreased apoptosis and adverse clinical outcome in human glioblastoma in vivo. However, the therapeutic potential of targeting the PI3K/Akt/mTOR cascade by kinase inhibitors for apoptosis sensitization of glioblastoma has not yet been investigated in detail. Here, we report that inhibition of PI3K by the broad range PI3K inhibitor LY294002 or a class I selective PI3K inhibitor broadly sensitizes glioblastoma cells for both death receptor- and anticancer drug-induced apoptosis. PI3K inhibitors synergize with TRAIL, agonistic anti-CD95 antibodies and various chemotherapeutic agents, e.g. temozolomide, doxorubicin, taxol, vincristin and VP16, to trigger apoptosis and also to suppress clonogenic growth in longterm survival assays. In contrast to PI3K inhibition, blockade of the PI3K/Akt/mTOR cascade at the level of mTOR by RAD001 or rapamycin does not enhance the sensitivity of glioblastoma cells to TRAIL- or doxorubicin-induced apoptosis. Similar to pharmacological inhibitors, genetic knockdown of PI3K subunits p110alpha and/or p110beta by RNA interference primes glioblastoma cells for TRAIL- or doxorubicin-mediated apoptosis, whereas silencing of mTOR does not alter apoptosis sensitivity. Analysis of apoptosis pathways reveals that inhibition of PI3K cooperates with TRAIL or doxorubicin to trigger loss of mitochondrial membrane potential, release of cytochrome c from mitochondria and full activation of the caspase cascade. Inhibition of caspases by the broad range caspase inhibitor zVAD.fmk completely abolishes apoptosis in response to the combination treatment, demonstrating that PI3K inhibitors sensitize for apoptosis in a caspase-dependent manner. Interestingly, PI3K inhibitors significantly enhance DNA damage caused by chemotherapeutic drugs and also reduce DNA repair, providing a molecular explanation for the synergistic killing of glioblastoma cells by PI3K inhibitors combined with DNA-damaging drugs. Most importantly, PI3K inhibitors prime patients' derived, primary glioblastoma cells for TRAIL- or chemotherapy-induced cell death. By demonstrating that PI3K inhibitors enhances both death receptor- and anticancer drug-induced apoptosis in glioblastoma cell lines and also in primary glioblastoma samples, our findings have important implications for the development of new treatment strategies for glioblastoma. Thus, PI3K inhibitors represent a promising novel approach to enhance the antitumor activity of TRAIL or chemotherapy in glioblastoma.

Proteasome

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Profiling of cancer cell signaling pathways activated by a novel proteasome inhibitor class (syrbactins) in human neuroblastoma

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Background: Syringolin A (SylA) and glidobactin A (GlbA) are plant pathogen-produced natural products and belong to a novel structural class of proteasome inhibitors (syrbactins) (Nature, 2008, 452:755−8). This study aimed to elucidate the signaling molecules and associated pathways activated in human neuroblastoma cells in response to SylA and GlbA treatments.
Material and Methods: The human neuroblastoma cell line SK-N-SH was grown in cell culture dishes in the presence of RPMI 1640 medium, 10% FBS, and antibiotics. Sub-confluent cells were treated with SylA (25 μM), GlbA (0.25 μM) or controls (water and DMSO, respectively). After 24 hours,