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The effect of carbohydrate- and fat-restricted diet on B16F10 melanoma model in C57BL6 mice: focus on tumor growth inhibition and its underlying molecular target signaling pathways

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Background: In cancer metabolism, cancer cells have higher aerobic glycolysis than that of normal cells even when oxygen is present. Increased glucose uptake and glycolysis in cancer cells have been shown to correlate with poor prognosis, increased invasiveness and metastatic potential in numerous cancers. Recent evidence reported that dietary restriction of carbohydrate and energy supplies may influence various cancer cells metabolism and their growth delay. However, the effect of those nutrient restrictions and their underlying signaling mechanisms are not well understood.
In our study, we tested whether restriction of dietary carbohydrate and fat (CFRD), without energy restriction relative to comparison diets, would slow tumor growth and reduce the molecular survival signaling pathway in B16F10 mice melanoma which is generally chemoresistant and carries poor prognosis.
Material and Methods: Male C57BL6 mice were randomly divided into two groups (n = 15/group) and fed experimental diet as follows: CFRD (20% carbohydrate, 2% fat, 78% protein, including ingredients supplementation) and regular diet as control. Following a preliminary feeding period for one week, mice were subcutaneously injected with 1×10^6 B16F10 cells and tumor volumes were measured for 3 weeks and sacrificed. Tumor tissues were excised for western blot and microarray analysis for the evaluation their molecular mechanisms of action and differences in gene expression.
Results: CFRD fed mice had decreased tumor volume and prolonged survival by up to 47% and 33.4%, respectively. In order to examine the mechanism of action of CFRD in the melanoma xenograft model, the expression pattern of several intracellular signaling molecules related to energy metabolism within the excised melanoma tissues were evaluated. Higher expression levels of hexokinase (HK)-II, PI3K/*p*-Akt and mammalian target of rapamycin (mTOR) related signaling were significantly attenuated by CFRD. We also observed increased expression of AMP-activated protein kinase (AMPK) related signaling proteins in CFRD mice tumor compared with control group. In addition, melanogenic-related genes and proteins such as DEK, MITF and TYRP-1 were decreased in CFRD mice.
Conclusions: Taken together, these findings suggested that CFRD not only delays or inhibits tumor growth, but also affects melanoma cell metabolism and survival signaling pathways in the both levels of protein and gene expression, resulting in the modulation of HK-II, AMPK, mTOR, Akt and melanogenic-related protein, which plays a key role in the survival of the melanoma cells. Overall, our study provides a rationale and experimental basis for using a combination of CFRD and chemotherapeutic drugs to improve treatment of melanoma cancers.

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Evaluation of the antitumor activity of pemetrexed in combination with the Chk1 inhibitor LY2603618

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The antifolate pemetrexed (Alimta®, PMX), which targets thymidylate synthase (TS), is currently approved by FDA and EMEA for the treatment of selected indications of advanced or metastatic NSCLC of nonsquamous histology. Inhibition of cellular de novo thymidine nucleotide synthesis is known to induce cell cycle arrest and checkpoint activation. The combination of PMX with the checkpoint kinase 1 (Chk1) inhibitor LY2603618 is currently undergoing phase 2 clinical trial. Here we report its preclinical activity in NSCLC tumor models. Treatments of subcutaneous xenografts implanted in female athymic nude mice via intraperitoneal route were initated when tumor volumes averaged 100 mm^3. Antitumor effects were assessed on day 67 using ratios of the average tumor volumes of treated (T) to vehicle control (C) group in percent (=100X[T/C]). In the H2122 xenograft model, PMX alone at 100 mg/kg given at q3dx15 caused T/C = 73%. PMX at this dose and schedule combined with LY2603618 at 45 or 90 mg/kg given 24 h after each PMX injection resulted in T/C = 52% ($p = 10^{-3}$) or 32% ($p < 10^{-3}$), respectively. LY2603618 alone at 45 mg/kg was inactive, whereas 90 mg/kg produced T/C = 82%. The H441 tumor model showed a similar inhibitory trend. H2122 tumors were harvested at 2 h after q3dx4 of the combination or LY2603618 alone, and 26 h after PMX alone. Western analysis showed that PMX alone at 100 mg/kg induced a 2.8 fold ($p < 10^{-3}$) increase in autophosphorylation of Chk1 S296 (pChk1) over vehicle. Combined with LY2603618 at 90 mg/kg reduced this increase by 29% to 2.0 fold ($p < 10^{-3}$), whereas LY2603618 alone had no effect. H2122 cultured in RPMI 1640 with 10% dialyzed fetal bovine serum exposed for 6 days to PMX or LY2603618 alone at 100 nM inhibited cell growth by >90% ($IC_{50} = 2$ nM) or <25% ($IC_{50} = 271$ nM) respectively. Compared to vehicle, 100 nM PMX alone at 28 h induced a 14× ($p < 10^{-3}$) increase in pChk1. While LY2603618 alone had no effect, a combination involving 100 nM PMX for 21 h followed by additional 7 h with 100 nM LY2603618 induced 7.6X ($p < 10^{-3}$) increase in pChk1 over vehicle, representing a 46% reduction from PMX alone. However, pChk1 induced by PMX alone was less abated when PMX+LY2603816 were added concurrently for 28 h, or 2 h LY2603618 prior to further inclusion of PMX for 28 h total. In conclusion, compared with PMX alone, sequential treatment of PMX and LY2603618 showed enhanced antitumor activity, which could be due to interference of the PMX induced cell cycle checkpoint by LY2603618.

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Antitumor efficacy of novel hedgehog inhibitors

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Background: The Hedgehog (Hh) signaling pathway plays an important role in tissue growth and repair. Aberrant activation of Hh pathway is involved in multiple tumor types. Previous studies have demonstrated potential synergy between Hh inhibitors and *nab*-paclitaxel (Abraxane, *nab*-P) in pancreatic cancer models. Hh-Gli signaling pathway has been shown to be essential for tumor growth, recurrence, and metastasis in the HT29 human colon carcinoma model. In this study, we tested the antitumor efficacy of a series of novel Hh inhibitors alone and in combination with *nab*-P against HT29 xenografts.
Material and Methods: A series of novel Hh inhibitors were synthesized and screened in vitro for inhibition of Gli1 activity. Selected compounds were tested in male athymic mice bearing HT29 xenografts at 25, 50, 75 and 100 mg/kg by IP, qdx12 or oral dosing, bidx12. *nab*-P was intravenously administered at suboptimal dose of 10 mg/kg, q4dx3.
Results: Hh inhibitors as single agents showed modest antitumor activity. In combination, Hh inhibitors by IP or bid oral dosing significantly increased antitumor efficacy of *nab*-P. In particular, ABI-2088 consistently demonstrated superior activity and lower toxicity compared with the known Hh inhibitor GDC 0449 (Curis/Genentech) when combined with *nab*-P. ABI-2088 (100 mg/kg, bidx12, PO) + *nab*-P resulted in TGI of 91%, significantly better than *nab*-P alone (TGI 66.5%, $P < 0.001$) and GDC 0449 + *nab*-P (TGI 81.4%, $P < 0.01$).
Conclusions: A number of novel Hh inhibitors were synthesized and screened for activity in vitro and in vivo. Oral delivery of these compounds in combination with *nab*-P showed synergistic activity and promise as anticancer agents.

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Perifosine in combination with antimetabolites induces synergistic effects on cytotoxicity and apoptosis in human colon, multiple myeloma, breast, renal, and liver tumor cell lines

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Perifosine, a novel, potentially first-in-class, oral Akt inhibitor, is currently in phase III trials for advanced colorectal cancer and multiple myeloma, as well as in other phase I and phase II trials for several other tumor types. To explore novel treatment options we evaluated the synergistic potential of Perifosine in combination with selected antimetabolites in a broad panel of human cancer cell lines.
Measurement of the cellular cytotoxic/antiproliferative activity of Perifosine w/wo combination with different antimetabolites was based on the dye Resazurin, which exhibits fluorescence change relating to cellular metabolic reduction (Nociaro et al. 1993, Int. J. Oncology 3, 473). The analysis of drug combinations was conducted by using the CalcuSyn software (Biosoft, Cambridge, UK), which yielded the combination index (CI), dose reduction index (DRI) and an isobologram analysis as classification (T.C. Chou 2006, Pharmacological Reviews 58, 621–681). Perifosine and representative antimetabolites were further evaluated regarding their effects on cell cycle and induction of apoptosis.
Synergistic cytotoxic/antiproliferative activity was demonstrated for Perifosine in combination with various antimetabolites in human colon,