apoptosis. Our results suggest that MEIS2 is direct target of HDACi in SS and supports a mechanism of cell killing through re-activation of repressed genes driving mesenchymal differentiation.

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Localization of human TACC3 to centrosomes is mediated by phosphorylation on serine 558 by aurora a; a novel pharmacodynamic method for measuring aurora a activity

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Aurora A is a serine/threonine protein kinase essential for normal mitotic progression. Aberrant increased expression of Aurora A, which occurs frequently in human cancers; results in mitotic spindle defects, chromosome instability and possibly tumorigenesis. Aurora A localizes to centrosomes and the proximal mitotic spindle during cell division and phosphorylates a variety of microtubule-associated proteins, including TACC3. TACC3 forms a complex at the centrosomes with ch-TOG where it modulates microtubule stabilization of the mitotic spindle. Recent studies identified a conserved serine in Xenopus (Ser626) and Drosophila (Ser863) TACC3 orthologs that is phosphorylated by Aurora A. We demonstrate that this conserved serine on human TACC3 (Ser558) is also phosphorylated by Aurora A *in vitro*. Moreover, phosphorylation of TACC3 by Aurora A is essential for its' proper localization to centrosomes and proximal mitotic spindles. Exogenously expressed wild type TACC3, but not Ser558 to Ala558 mutant, localize to centrosomes in cultured human tumor cells. Inhibition of Aurora A in cultured human tumor cells with the selective small molecule inhibitor MLN8054 results in mislocalization of endogenous TACC3 away from centrosomes in a dose-dependent manner. Furthermore, oral administration of MLN8054 to mice bearing human tumor xenografts also disrupted TACC3 localization to centrosomes detected in tumor sections. In summary, this work introduces a novel pharmacodynamic method for measuring Aurora A activity by quantifying the loss of TACC3 from centrosomes and proximal mitotic spindles.

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The anticancer agent, ECO-4601, is a potent inhibitor of the Ras-mitogen-activated protein kinase pathway

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Background: ECO-4601 (MW 462), a farnesylated dibenzodiazepinone, is a promising new chemical entity discovered through Ecopia's Decipher® technology, a proprietary drug discovery platform. The compound was shown to have a broad cytotoxic activity (low μM range) when tested in the NCI 60 cell line panel. Antitumor evaluation in human glioma, breast and prostate xenografts indicated that ECO-4601 had potent antitumor activity (EORTC-NCI-AACR 2005, Abstract #2910). Although the compound binds the peripheral benzodiazepine receptor (PBR) (AACR 2006, Abstract # 5896), transcriptome analysis and antitumor data suggest that other mechanisms are involved. Related to its farnesylated moiety, the effect of ECO-4601 was assessed on the Ras signaling pathway.
Material and Methods: We first verified if ECO-4601 interfered with Ras processing by monitoring farnesyltransferase (FPTase) and geranylgeranyl transferase (GGPTase I) activities. Downstream Ras signaling events, such as Raf-1 and ERK1/2 phosphorylation, were also evaluated by immunoblots in prostate (PC-3), breast (MCF7 and MDA-MB-231) and glioma (U-87 MG) cell lines. Cells were treated with 10 μM ECO-4601 for 30 min, 1, 4 and 6h. Subsequently, half of the treated cells were exposed to EGF (50 ng/ml) for 10 min.
Results: No mobility shift of either HDJ2 or Rap1A (specific surrogate markers of FPTase and GGPTase I, respectively) were observed in PC-3 or MCF7 cells exposed to ECO-4601 for up to 48h. In contrast, a strong inhibition of EGF-induced phosphorylation of *c-Raf-1* and *ERK1/2* in the four cell lines tested was shown. This effect was time dependent with *complete inhibition* of protein phosphorylation within 6 h.
Conclusions: Our data suggest that ECO-4601 is a potent inhibitor of the Ras-mitogen-activated protein kinase pathway. The inhibitory activity appears to be prior to Raf-1 phosphorylation and post prenylation. ECO-4601 is presently being tested in a Phase I clinical trials against solid tumors.

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Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma

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Background: Sorafenib is an oral multi-kinase inhibitor with anti-angiogenic and anti-proliferative activity that targets the Raf/MEK/ERK pathway at the level of Raf kinase and receptor tyrosine kinases VEGFR-1/-2/-3 and PDGFR-. Sorafenib has demonstrated efficacy against several tumor types, including hepatocellular carcinoma (HCC), in Phase I/II trials. This Phase I trial was conducted to evaluate the pharmacokinetics (PK), safety and tolerability, and preliminary efficacy of sorafenib in Japanese HCC patients with underlying liver dysfunction.
Material and Methods: Patients with histologically confirmed, unresectable HCC, Child–Pugh status A or B, and adequate organ functions were treated. A single dose of sorafenib was administered, followed by a 7-day wash-out period. After the wash-out period, patients received either sorafenib 200 mg (Cohort 1) or 400 mg (Cohort 2) twice daily (bid) for 28 days (Cycle 1). From Cycle 2 onwards, patients continued sorafenib until disease progression or intolerable toxicity. The tolerability at each dose level was correlated with Child–Pugh class. Efficacy was evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST).
Results: A total of 27 patients were enrolled; 24 patients were evaluable for PK, tolerability, and efficacy. Despite a relatively high level of interpatient variability in PK, both AUC_{0-12} and C_{max} at steady state were slightly lower in Child–Pugh B patients. Common adverse drug toxicities included elevated lipase (85.2%), rash/desquamation (40.7%) and hand–foot skin reaction (HFSR; 33.3%). A dose-limiting toxicity of HFSR was observed in one patient (Cohort 2). One patient (4%) achieved a partial response (PR), 20 (83%) had stable disease, and three (13%) had progressive disease. The patient who experienced PR continued sorafenib treatment for $\geqslant$1 year. For the 27 patients, median progression-free survival was 4.9 months and median overall survival was 15.6 months.
Conclusions: Sorafenib demonstrated favorable tolerability in Japanese HCC patients. There were no clinically relevant differences in PK and safety between Child–Pugh A and B patients. Further studies of sorafenib are warranted in HCC, based on its encouraging safety profile and preliminary anti-tumor activity.

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COX-2 expression by Wnt signaling activation decides radiosensitivity of head and neck cancer in association with regulation of Ku 70/80

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Background: Ku70/80 is an important molecule in repair of DNA damage by irradiation to head and neck cancer. However, little is known of molecular mechanisms regulating Ku 70/80 expression. We examined role of Wnt signaling and Ku70/80 expression and their associations with cyclooxygenase-2 (COX-2) in the radioresistance mechanism of head and neck cancer.
Material and Methods: COX-2, β-catennin, and Ku 70/80 changes before and after irradiation to a head and neck cancer cell line, AMC-HN3, having moderate radiosensitivity, were examined by immunflurescence, western blotting, and real-time PCR in a condition of pretreatment with by 1 μM (2′Z,3′E)-6-bromoindirubin-3′-oxime (BIO). By BIO treatment following 4-Gy irradiation, viability change and radiosensitivity of the cancer cells were analyzed by FACS and clonogenic assays. Radiosensitivity of the cancer cells after transfection with COX-2 siRNA or celecoxib, a selective COX-2 inhibitor was observed by the change of Ku70/80 by western blotting.
Results: Activation of Wnt signaling pathway, increased β-catennin level, by BIO treatment resulted in the increased expression of COX-2 and Ku 70/80, increasing radioresistance of the irradiated cancer cells. COX-2 suppression by siRNA or celecoxib induced no significant change Ku70/80 level in the condition of Wnt signaling activation by pretreatment of BIO and recovered the radiosensitivity of the cancer cells, suggesting that COX-2 may play a role in the radioresistance mechanism by Ku70/80 induction.
Conclusions: COX-2 expression by Wnt signaling activation is a key molecule in regulating Ku70/80 induced by radiation and thus, in the radioresistant mechanism. This may include a therapeutic implication that the suppression of Ku70/80 or COX-2 contributes to increasing the radiosensitivity of head and neck cancer.