

Materials and Methods: The tumour was a C3H mammary carcinoma implanted in the right rear foot of female CDF1 mice and treated when at 200mm³ in size. Oxi 4503 was dissolved in saline immediately prior to each experiment and injected intraperitoneally at doses of 50–250 mg/kg either as single or multiple (once weekly for upto 4 weeks) treatments. Radiation (240 kV x-rays) was given as single doses locally to tumours. Tumour response was estimated using either tumour growth time (TGT; time for tumours to grow from 200 to 1000 mm³), or local tumour control (LTC; percentage of mice showing tumour control 90 days after treatment) and following logit analysis of the radiation dose response curves the TCD50 value (radiation dose producing 50% tumour control) calculated. Statistical analysis was performed using either a Student's t-test (TGT) or Chi-squared test (LTC), with the significance level being $p < 0.05$ in both cases.

Results: The mean (± 1 S.E.) TGT for control tumours was 6.3 (6.1–6.5) days. This was significantly increased following injection of 50, 100 or 250 mg/kg Oxi 4503. The respective TGTs being 8.8 (8.4–9.3), 9.1 (8.7–9.5) and 10.3 (10.0–10.6) days with single treatments, and 13.5 (13.2–13.8), 19.5 (17.6–21.4) and 28.1 (25.8–30.3) days with multiple treatments. The TCD50 value ($\pm 95\%$ confidence limits) for radiation alone was 53 Gy (51–55). A single treatment with 50 mg/kg Oxi 4503 administered 30 minutes after irradiating significantly reduced this to 41 Gy (38–45). This was further significantly decreased to 34 Gy (31–38) when the same Oxi 4503 dose was given in a multiple schedule.

Conclusions: Unlike other tubulin-binding drugs Oxi 4503 has substantial anti-tumour activity when given as a single agent, this effect being dependent on the total drug-dose administered and how often it was given. Oxi 4503 also enhanced the tumour response to radiation when given either as a single drug treatment or in a repeated multiple-drug dosing schedule. The enhancement seen with the latter approach was greater than that seen with any other clinically relevant treatment combined with radiation in this tumour model.

Supported by a grant from the Danish Cancer Society

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POSTER

Signaling dependent phosphorylation of tubulin folding factor controls microtubule biogenesis and functions: a new potential target in cancer cells

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Background: Microtubules (MT) are critical regulatory components of normal cellular processes such as chromosomal segregation, morphogenesis, organelle positioning and cell migration. MTs are also involved in pathological conditions such as the formation of multiple microtubule-organizing centers (MTOC), an accepted indicator of aneuploidy in cancer cells. We investigated that Pak1 may influence MT dynamics and functions through regulatory targeting of critical tubulin cofactor phosphorylation and function. **Material and Methods:** A yeast two-hybrid screening of human mammary gland cDNA expression library using Pak1 as bait identified tubulin cofactor (TCoF) as a Pak1-interacting protein. **Results:** Here we identified TCoF, an essential component of α -tubulin folding and MT biogenesis, as a substrate of Pak1. Pak1 but not Pak2 directly phosphorylated TCoF both in vitro and in vivo, and co-localized with the endogenous TCoF on newly synthesized microtubules as well as on centrosomes and mitotic spindles. TCoF interacted with the GTPase binding domain of Pak1 and activated Pak1 in both in vitro and in vivo assays. Pak1 and its upstream activators phosphorylated endogenous TCoF at two sites, serine 65 and serine 128. Transfection of a Ser65, 128Ala TCoF mutant protein as well as siRNA-mediated knock down of either endogenous TCoF or Pak1 inhibited microtubule biogenesis, suggesting that Pak1 phosphorylation is necessary for normal TCoF function and that TCoF is critical for microtubule assembly. **Deregulation of TCoF in human epithelial cells expressing functional endogenous Pak1 resulted in a dramatic increase in the number of g-tubulin-containing MTOC. Further, Pak1 and TCoF were co-deregulated in human breast tumors. Conclusion:** Our results show that Pak1 phosphorylates and regulates the functions of the microtubule assembly protein TCoF. We have discovered an unrecognized role of Pak1-dependent signaling pathways in microtubule biogenesis and functional dynamics. This regulatory interaction directly influences the number and function of MTOC of the cell, which determines the number, polarity, and organization of interphase and mitotic microtubules. Since TCoF deregulation in mammalian cells with functional Pak1 increased the number of MTOC, and since TCoF and Pak1 are deregulated in human breast tumors, these findings implicate a mechanistic role of Pak1-TCoF in the development of aneuploidy and associated genomic instability in human cancers.

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POSTER

The seco-taxane idn5390 is able to target class III beta-tubulin and to overcome paclitaxel resistance

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A prominent mechanism of drug resistance to taxanes is the overexpression of class III beta-tubulin. Levels of beta-tubulin isotypes by RT-PCR were assessed in cells treated with paclitaxel, IDN5390, a new taxane-derivative, and with their combination. In wt cells, paclitaxel was able to increase class III beta-tubulin levels, whereas IDN5390 induced the opposite effect, and the combination paclitaxel/IDN5390 prevented class III overexpression. In paclitaxel-resistant cells, which express a high level of class III beta tubulin, IDN5390 alone and in combination diminished the expression of the class III. Moreover, the combined treatment paclitaxel/IDN5390 yielded a strong synergism. The strong synergism paclitaxel/IDN5390 was also evident in cell-free tubulin polymerisation assays. In the presence of an anti class III beta-tubulin as blocking antibody, tubulin polymerisation induced by paclitaxel and IDN5390 was enhanced and not affected, respectively, whereas synergism was abolished, thereby indicating IDN5390 activity is not modulated by class III beta-tubulin levels. Such properties can be explained taking into consideration structure of class III beta-tubulin paclitaxel binding site: in fact, Serine 276 interacting with C group of paclitaxel in class I is replaced by an Arginine in class III. IDN5390 that has an open and flexible C ring with an extra negative charge better fits than paclitaxel with class III beta tubulin at the paclitaxel binding site. Taking altogether, these findings indicate that the concomitant treatment IDN5390/paclitaxel is able to target successfully class I and III beta-tubulin and could represent a novel approach to overcome paclitaxel-resistance.

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POSTER

ABT-751 enhances the efficacy of 5-FU, cisplatin and gemcitabine in preclinical xenograft models

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ABT-751 is an orally active antimitotic agent that is currently in Phase II clinical trials. This agent binds to the colchicine site on β -tubulin and inhibits polymerization of microtubules. This disruption of microtubule dynamics leads to a block in the cell cycle at the G₂/M phase resulting in the induction of cellular apoptosis. The oral bioavailability of ABT-751 in mouse, rat, dog and monkey ranged from 33.0 to 87.7% with bioavailability highest in the mouse. ABT-751, as a single agent demonstrated antitumor activity against a series of xenograft models including colon, NSCLC, breast, and pancreas. The current studies were conducted to determine whether ABT-751 enhances antitumor activity of standard cytotoxic therapies currently in clinical use. Efficacy of ABT-751 in combination with 5-FU, cisplatin and gemcitabine was evaluated in the HT-29 colon, Calu 6 NSCLC and MiaPaCa-2 pancreatic xenograft models, respectively. HT-29 tumor-bearing nude mice were treated with ABT-751 orally once-a-day at 100 mg/kg/day on a 5 days on, 5 days off schedule for 2 cycles, in combination with maximally tolerated dose (MTD) 5-FU, 30 mg/kg/day, i.p., q.d.x5. The %T/C ratios on day 38 were 22, 28, and 5 and the %ILS values were 75, 75, and 150 for 5-FU, ABT-751, and 5-FU + ABT-751 treated groups, respectively. Efficacy of ABT-751 at the same dose and schedule was tested in combination with MTD of cisplatin (10 mg/kg/day, i.p., $\times 1$) in Calu-6 tumor-bearing nude mice. The %T/C ratios on day 38 were 56, 37 and 6 and the %ILS values were 58, 65 and 188 for cisplatin, ABT-751 and the combination groups, respectively. ABT-751 at 100 mg/kg/day (p.o., q.d., for 2 cycles) was evaluated in combination with gemcitabine administered at 20 mg/kg/day on a q3dx4 schedule (1/6th of MTD) against MiaPaCa-2 human pancreatic cancer xenografts grown in the orthotopic site. ABT-751 enhanced efficacy of gemcitabine. The %T/C ratios on day 29 were 56, 82 and 33 for ABT-751, gemcitabine and the combination groups, respectively. Phase I pharmacokinetic data indicate that ABT-751 is well-absorbed and achieved plasma concentrations that were efficacious in preclinical models. Collectively, these studies demonstrate that ABT-751 enhanced efficacy of standard cytotoxic therapies at their MTDs with no overlapping toxicities.