



Title	Inhibition of tumour growth by Raf265 via blockade of Raf/MEK/ERK pathway in colorectal cancer
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Introduction: Deregulation of the Raf/MEK/ERK signalling pathway is commonly observed in colorectal cancer (CRC). Since this signalling pathway plays a central role in controlling cell proliferation, apoptosis and differentiation; therefore, a number of therapeutics targeting on the Raf/MEK/ERK pathway has been established recently. Raf265 is an orally bioavailable small molecule which is a potent inhibitor of wild-type and mutant (eg V600E) B-raf kinases. The study of the effect of Raf265 has entered phase I clinical trial in subjects with locally advanced or metastatic melanoma. However, its effect in CRC has not yet been fully understood. The objective of this study was to examine the functional effects of Raf265 in CRC cells in vitro and in vivo.

Methods: Colorectal cancer cell lines of different B-raf status were used in this study. Cell proliferation upon Raf265 treatment (0-50 μ M) was determined using 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay. Cell cycle distribution and cell apoptosis upon Raf265 treatment (0, 1, 5, 10, and 15 μ M) were assessed by flow cytometry. Phosphorylation of molecules including MEK and ERK, and eIF4E, and expression of Mcl-1 and cyclin D1 was analysed using western blot. For in-vivo animal studies, subcutaneous tumours were established by subcutaneous injections of 1×10^6 cells into nude or SCID mice, and tumour growth was monitored. Mice were sacrificed at week 16 or when tumour sizes exceeded 30% of their body weight.

Results: Raf265 was found to significantly inhibit cell proliferation in a dose-dependent manner with IC_{50} at 0.83 to 5.54 μ M. Increased annexin V positive cells were observed with escalating dose of Raf265, which is indicative of induction of apoptosis in CRC cells. Dose-dependent increase in G_1 and decrease in S phase population (cell cycle arrest at G_1 phase), and increase in the 'sub- G_0 ' population was also observed after treatment with Raf265. This was accompanied by the reduction of phosphor-MEK and phosphor-ERK. Down-regulation of Mcl-1 and cyclin D1, which are genes regulating apoptosis and cell proliferation, was also observed. Intraperitoneal injections of Raf265 four times weekly demonstrated significant anti-tumour activity in established tumours of xenograft models. Immunohistochemistry demonstrated a close association between inhibition of tumour growth and inhibition of the extracellular signal-regulated kinases (ERKs) 1/2 phosphorylation in the xenograft tumours, consistent with inhibition of the RAF/MEK/ERK pathway. Additional analyses of microvessel density and microvessel area in the same tumour sections using antimurine CD31 antibodies demonstrated significant inhibition of neovascularisation in xenograft models.

Conclusions: These pre-clinical data demonstrate robust anti-tumour activity of Raf265, providing the basis for exploiting its potential use as a therapeutic for Raf-driven CRC tumours.

Bioavailable testosterone predicts a lower risk of Alzheimer's disease in older men: a 1-year cohort study

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Introduction: There are limited data on the protective effects of testosterone regarding Alzheimer's disease (AD) in older men. The objective of this study was to investigate the protective effects of serum total (TT), bioavailable testosterone (BT), and sex hormone binding globulin (SHBG) levels on the subsequent risk of AD in non-demented Chinese older men.

Methods: This 1-year prospective cohort study was carried out in an ambulatory setting. The subjects were ambulatory community-living non-demented Chinese older men. Morning serum TT, BT and SHBG levels were determined for all subjects at baseline, and 1-year prospective follow-up assessment for dementia and AD were done. Alzheimer's disease was diagnosed by the NINCDS-ADRDA criteria for probable AD and aMCI by the Petersen's criteria.

Results: A total of 153 older men (83% of baseline subjects) completed the 1-year follow-up study. Their mean age was 72.7 years. 6.5% (n=10) developed dementia (converters), all having AD. 93.5% (n=143) did not develop dementia (non-converters). Logistic regression analyses for independent predictors of AD showed that the baseline serum BT level, systolic blood pressure (SBP) and ApoE ϵ 4 genotype were significant independent predictors, after adjustment for age, education, body weight, body mass index, fasting plasma glucose level, serum HDL-C and SHBG levels. The baseline serum BT level was protective against the development of AD, and the adjusted relative risk (RR) of BT was 0.22 (95% CI, 0.07-0.69). Baseline SBP and ApoE ϵ 4 genotype were independent risk factors, with RRs of 1.04 and 5.04 respectively.

Conclusion: Bioavailable testosterone in late life protects against future AD development in older men.

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