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Drug 10 and A inhibited transcription of HIF-1 downstream target genes by affecting its transactivation function

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Under the condition of low oxygen level (hypoxia, anoxia), hypoxia inducible factor-1 (HIF-1) is stabilised and induces transcription of around 100 downstream target genes, many of which play important roles in tumorigenesis, such as VEGF-1 for angiogenesis, LDH-A and GLUT-1 for metabolism, and LOX for metastasis. HIF-1 also positively regulates chemo- and radio-resistance of cancer cells. In clinical studies, HIF-1 expression in tumour biopsies is correlated with poor prognosis. Taken together, these data suggest that inhibiting HIF-1 transactivation would be beneficial for cancer therapy.

Previously we generated a HCT116 human cancer cell line, which stably expresses a firefly luciferase reporter and a renilla luciferase reporter under the control of a hypoxia response element (HRE) of the LDH-A gene and general transcription promoter respectively (HCT116dc cell). By using HCT116dc cells as a high-throughput screening method, we have initially identified a compound, designated as Drug 10, as a potent HIF-1 inhibitor. Derivatives of Drug 10 were synthesized for structure-function study, and Drug 10 and one of its derivatives Drug A, were found to be the most potent HIF-1 inhibitors among this series of compounds.

To unveil the mechanism of HIF-1 inhibition, further investigation was carried out. Firstly, to validate the Drugs' inhibition of HIF-1 transactivation, mRNA levels of HIF-1 downstream target genes were evaluated in both HCT116dc and HCT116 wild type (HCT116wt) cells. In anoxia (<0.01% O_2), mRNA levels of VEGF-1, LDH-A, and GLUT-1 increased, however these levels decreased when cells were treated with Drug 10 and A.

Secondly, to examine whether the inhibition was only restricted to HCT116 cells, HT1080wt cells were transient transfected with HRE-firefly luciferase reporter using adenovirus, so that HIF-1 transactivation function can be analysed by evaluating luciferase level, which is normalised by protein concentration. Drug 10 and Drug A both inhibited induction of firefly luciferase by anoxia in HT1080wt cells. This indicated that these inhibitors' effect was not just specific on HCT116 cells.

Thirdly, mRNA and protein levels of HIF-1α in HCT116wt cells were assayed. The mRNA level of HIF-1α had a substantial level in air and was not changed by anoxia. Drug 10 and Drug A had no effect on the mRNA level of HIF-1 in either air or anoxia condition. Besides, Western blot result showed that anoxia induced HIF-1α protein level however Drug 10 or Drug A did not decrease it. All of these data suggest that Drug 10 and Drug A interfered with HIF-1 transactivation but did not affect synthesis or stability of HIF-1α.

Drug 10 and Drug A were verified as inhibitors of HIF-1 function. They decreased transcription of HIF-1 downstream target genes induced by anoxia. This was not through decreasing HIF-1α mRNA or protein levels in anoxia but affecting on HIF-1 transactivation.

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A role for the Akt/mTOR pathway in the increased tumor growth of high-fat diet-induced obesity mice

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Diseases such as Obesity, Type II Diabetes Mellitus and Metabolic Syndrome are correlated to an elevated predisposition in some types of cancer, to aggressiveness increase in others and share a common state of hyperinsulinemia. mTOR signaling pathway is associated with carcinogenesis and is also the main insulin signaling pathway. However, the molecular mechanisms involved in the increased aggressiveness of prostate cancer in obesity needs to be better defined. Here we show the effects of diet induced obesity on the tumor growth and characterize the IRS/PI3-kinase/Akt/mTOR pathway in PC-3 and DU145 xenografts on SCID mice. Our results show that high-fat diet-induced insulin resistant mice had a larger tumor growth compared to the control group. We also noticed an increase in IRS-1 phosphorylation, IRS-1/PI3K association, Akt phosphorylation and mTOR activity in the PC-3 and DU145 xenografts after acute insulin treatment on the high-fat diet animals, however in these animals the activation of this signaling pathway was reduced in peripheric tissues involved in insulin sensitivity (liver and muscle). Our data suggest that the IRS/PI3-kinase/Akt/mTOR pathway directs the metabolic signals to tumor growth and that the increase in the activation of this signaling pathway is involved in the aggressiveness increase of the prostatic tumors in diet induced obesity.

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Chronic treatment with irinotecan activates the PI3K/Akt/mTOR pathway in HT-29 colon cancer xenografts

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Resistance of tumors to chemotherapeutic agents is a common clinical problem in human cancer. Recently, the blocking of the PI3K signaling pathway was shown to enhance apoptosis induced by SN-38, an active form of irinotecan. To gain further insight into the molecular events of the irinotecan-associated increase in the PI3K signaling pathway, aspirin and rapamycin were used to modulate this signaling pathway. We herein report that aspirin is able to further inhibit IRS-1 serine phosphorylation induced by irinotecan through targeting of JNK and NFkB. Thus, agonist activation of the IRS-1/PI3K pathway blocked the growth-inhibitory effect of irinotecan in HT-29 colon cancer xenografts; our data also demonstrate a synergistic effect of mTOR inhibition and irinotecan on tumor growth. Activation of the PI3K/Akt/mTOR pathway may, thus, contribute to refractoriness for treatment with irinotecan.

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Down-regulation of p53 protein expression in lung carcinoma cells in response to the tyrosine kinase inhibitor gefinitib

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Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have been shown to produce dramatic responses in a fraction of non-small cell lung cancer patients. Over the past years, clinical and biological predictors for TKI sensitivity have been identified. Among clinical features, never-smoking history seems the most critical factor, probably because of the different spectrum of molecular abnormalities associated with cigarette smoking exposure. However, clinical responses to TKI are at best transient and the general impact of TKI on overall cancer survival is limited. Among biological predictors, several studies indicate that EGFR mutations and increased EGFR gene copy number are implicated in response to TKI therapy, with conflicting results on survival. Second mutations in the EGFR gene, as well as in K-RAS, impair TKI effects, leading to TKI resistance. In a previous study, we have observed that p53 function is systematically disrupted in primary lung carcinomas with EGFR activating mutation. This inactivation can take place either through TP53 mutation, or through loss of expression of p14arf, or both. P14arf is a critical regulator of the anti-proliferative response to excessive or untimely growth stimuli by controlling the activity of Hdm2, which itself controls p53 stability. These results suggest that p53 is a rate-limiting factor for cell proliferation induced by activation of the EGFR, and that cells with a constitutively active receptor must abrogate p53 function in order to prevent growth suppression or apoptosis. To further investigate the role of p53 in the cascade of signaling events dowstream of EGFR, we have investigated the effects of tyrosine kinase inhibitors on p53 expression function in lung cancer celll lines with different TP53 and EGFR mutation status. We show that inactivation of EGFR kinase with selective inhibitors significantly reduces p53 expression by down-regulating p53 mRNA. This transcriptionnal repression is mediated through NfkappaB which negatively controls p53 promoter. Our findings suggest that downregulation of p53 may represent an adaptative mechanism that allow cell survival upon TKI and ultimatly promotes escape from therapy and tumor relapse.

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Human non-small cell lung cancer (NSCLC) cell lines with inactivated LKB1 and KRAS mutations are sensitive to MEK inhibition

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LKB1 is a tumour suppressor kinase, germline mutations of which are responsible for Peutz-Jeghers syndrome a hereditary condition that leads