Methods: Dendritic nanocarriers were synthesized and further characterized by dynamic light scattering, atomic force microscopy and electrophoretic mobility shift assay. Cellular internalization of polyplexes was monitored by confocal microscopy. The nanocarriers cytotoxicity profile was assessed by XTT and red blood cells lysis assay. The luciferase gene, ectopically overexpressed in human glioblastoma cell lines was used as a model system and its silencing efficacy was measured. mCherry and luciferase-labeled glioblastoma and mammary adenocarcinoma mouse models were established. The silencing efficiency of the nanocarrier luciferase-siRNA polyplexes was followed up by non-invasive intravital bioluminescence imaging.
Results: All dendritic nanocarriers synthesized had a mean hydrodynamic diameter of 10−40 nm. The novel nanocarriers entrap siRNA, neutralize its negative charge in a dose-dependent manner and significantly improve its cellular uptake. PG-Amine exhibited the optimal silencing efficiency and safety profile in additional *in vitro* biocompatibility and efficacy tests. Therefore, it was selected for further evaluation and *in vivo* gene silencing efficacy studies. A significant gene silencing effect was accomplished *in vivo* in both human glioblastoma and murine mammary adenocarcinoma mouse models. Within 24 hours, 85% and 68% silencing was achieved following intratumoural and intravenous treatment respectively, as measured by intravital non-invasive imaging of photon flux bioluminescence. No significant weight loss occurred following intravenous administration of the siRNA-nanocarrier complexes.
Conclusions: We show a proof of concept for siRNA delivery using a luciferase-based model. We predict that *in vivo* silencing of an important cell growth and angiogenesis regulator as Akt1 in a selective manner will warrant this approach as a successful anticancer therapy.

206 Downstream signaling pathways determine resistance of cancer cells against novel irreversible ErbB-targeting drugs

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Epidermal growth factor (EGF) receptor-related antigens (EGFR, ErbB1−4, HER1−4) represent emerging drug targets in oncology. However, resistance against ErbB-targeting drugs occurs frequently in cancer patients. Drug-resistant cells may exhibit drug-refractory phosphatidylinositol 3-kinase (PI3K) or mitogen-activated protein kinase (MAPK) signaling, but the relative impact and contribution of these two downstream pathways to drug resistance are still controversially discussed. We examined the effects of the two very potent, irreversibly binding ErbB receptor tyrosine kinase inhibitors (RTKIs) pelitinib (EKB-569) and canertinib (CI-1033) on PI3K- and MAPK activity in ErbB RTKI-sensitive and ErbB RTKI-resistant breast and ovarian cancer cells. Western blot analysis revealed that ErbB phosphorylation was abrogated by the inhibitors in both drug-sensitive and drug-resistant cells, whereas AKT- and GSK3b phosphorylation were drug-dependently downregulated only in drug-sensitive cells. ErbB RTKI sensitivity did not correlate with expression of wildtype PTEN or PIK3CA, nor was it associated with drug-dependent silencing of ERK1,2 in the breast and ovarian cancer cell lines examined. Moreover, exogenous AKT, but not MEK, significantly induced drug resistance. Our data demonstrate that blocking AKT phosphorylation is essential and sufficient, whereas abrogation of ERK phosphorylation is not required for ErbB RTKI anticancer efficacy. AKT phosphorylation may thus be a useful biomarker of ErbB RTKI sensitivity in breast and ovarian cancer cells. Supported by 'Medizinisch-Wissenschaftlicher Fonds des Bürgermeisters der Bundeshauptstadt Wien' (#08037) and 'Initiative Krebsforschung', Medical University Vienna.

207 Cellular mechanism of a novel CDK9 inhibitor CDKI-71

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Introduction: Human cancer cells resist apoptosis by relying on sustained expression of anti-apoptotic genes. Targeting transcription and suppress these anti-apoptotic proteins seem a promising strategy for anti-cancer therapy. CDK9-cyclinT1 activates RNA transcription by phosphorylating the CTD of RNAPII. CDKI-71, a potent CDK9 inhibitor, has been identified via our in-house drug discovery program. This compound inhibits RNAPII transcription, and effectively induces apoptosis in cancer cells. Hereby we report the detailed mechanism of action of CDKI-71.
Methods: Kinase inhibition was measured by radiometric assay. MTT assay was performed against the human tumour cell lines and non-cancerous cell line. Apoptosis was detected by Annexin-V and PI double stains. Phosph-RNAPII Ser-2, Ser-5, total RNAP II, Mcl-1, Bcl-2, p53, MDM-2, PARP, XIAP and g-H2AX were detected by western blots.
Results: CDKI-71 inhibits CDK9/T1 and CDK7/H with the K_i values of 6 nM and 114 nM respectively, having a similar potency and selectivity profile to the clinic CDK inhibitor flavopiridol (K_i = 3 and 113 nM for CDK9 and CDK7 respectively). Treatment of A2780 ovarian cancer cells with 0.5 mM CDKI-71 for 24 h blocked the phosphorylation at Ser-2 of CTD RNAPII, confirming its cellular CDK9 inhibitory activity. CDKI-71 is a potent anti-proliferative agent with an average GI_{50} of 0.55 mM against 12 human tumour cell lines. Interestingly, this compound shows a significantly reduced toxicity in the non-cancerous MRC-5 cells with a GI_{50} value of 4.3 mM. In contrast, flavopiridol exhibits little selectivity in the cancer and non-transformed cell lines. Treatment of HCT-116 and A2780 cells with CDKI-71 for 24 h resulted in a dose-dependent activation of caspase 3/7 and induction of apoptosis. However little affects on MRC-5 cells were observed by the same treatment. Blockage of Mcl-1 protein expression and increased nucleate p53 accumulation were also identified. To investigate whether CDKI-71 induces p53 protein is a consequence of DNA damage response we measured the level of γ-H2AX, the phosphorylated Ser-139 of H2AX, using western blots. Treating both A2780 and MRC-5 cells with vary concentrations of CDKI-71 for 24 h no γ-H2AX protein was detected. In contrast, exposure of the cells with 0.2 μM flavopiridol resulted in highly elevated level of γ-H2AX protein.
Conclusions: CDKI-71 is a potent and selective CDK9 inhibitor possessing excellent anti-proliferative activity. The compound induces apoptosis selectively in cancer cells over the normal non-transformed cells. CDKI-71 can be developed as an anti-cancer agent.

208 Downregulation of antiviral genes in prostate cancers affects the response to the DNA methylation inhibitor 5-aza-2′-deoxycytidine

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Background: Mutations and polymorphisms in genes encoding antiviral proteins, notably RNASEL, are associated with an increased risk of prostate cancer. In addition, in many prostate cancers interferon response genes, like the prototypic MX1, are downregulated. We sought to elucidate whether changes in DNA methylation underlie this downregulation.
Experimental procedures and Results: Treatment with the DNA methylation inhibitor 5-aza-2′-deoxycytidine (aza-dC) for 3 days induced MX1 and several other interferon-responsive genes in the PC3 but not the LNCaP prostate cancer cell line. According to bisulfite sequencing, the major promoter of MX1 was unmethylated in either line. Induction of MX1 in LNCaP was restored neither by increasing the inhibitor concentration nor the length of the exposure. In contrast to PC3, LNCaP expresses significant levels of androgen receptor and contains mutant RNase L. Nevertheless, depleting LNCaP medium of steroids did not restore MX1 induction by aza-dC. Neither was MX1 inducibility in PC3 diminished by siRNA-mediated downregulation of RNase L. Intriguingly, compared to PC3, LNCaP cells also displayed a strongly muted response to exogenous interferon beta (8- vs. 90-fold), due to a 50-fold decrease in expression of JAK1, a protein kinase crucial for interferon signaling. Accordingly, interferon beta expression in PC3 became upregulated after 2 days of aza-dC treatment, preceding the increase in MX1. This suggests that aza-dC treatment elicits interferon production, JAK1 activation and induction of interferon-responsive genes like MX1 in PC3 cells. This sequence of events appears to be interrupted by lack of JAK1 in LNCaP. Downregulation of JAK1 is not a pecularity of this cell line, but was observed in a significant fraction of prostate cancer tissues as well.
Conclusions: Our data suggest that downregulation of interferon-responsive genes in prostate cancers is not caused by hypermethylation of their promoters, but occurs rather secondary to changes in interferon signaling pathways, such as JAK1 deficiency. Our findings have implications for the potential use of DNA methylation inhibitors in cancer therapy, in particular predicting that aza-dC will induce interferon responses in some prostate cancers, but not others. These differences are expected to affect the efficacy of aza-dC and other inhibitors of DNA methylation in this cancer type.