three dose groups. The amounts of ReoT3D dsRNA on day 14 p.i. were significantly higher in tumor than spleen tissues regardless of the initial dose ($P < 0.0001$). Interestingly, the level of ReoT3D dsRNA in biopsied tumor specimens obtained on day 2 p.i. inversely correlated with the subsequent tumor growth ($r^2 = 0.76$, $P = 0.002$ when compared with tumor growth on day 14).
Conclusions: A single IV administration of ReoT3D can result in substantial tumor growth delay in melanoma-bearing nude mice, and the extent of acute phase ReoT3D replication in tumor tissues appears to predict the subsequent tumor response.
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Autocrine activity of tumor-derived membrane vesicles

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Background: Actively growing tumor cells release membrane vesicles into extracellular milieu, and the rate of shedding increases in malignant tumors. Tumor-derived membrane vesicles (TMVs), enriched in most surface antigens and proteases derived from their originating cells, are nowadays gaining attention as important mediators of cell-to-cell communication facilitating processes such as angiogenesis and immune modulation with paracrine functions. Since TMVs have the potential to affect to tumor cells themselves, the role of TMVs in autocrine signals to tumor cells has been investigated.
Material and Methods: We isolated TMVs from SW480, human colorectal adenocarcinoma cells by ultracentrifugation onto sucrose cushion and iodixanol gradients. We characterized TMVs with Western blotting using membrane vesicles markers, density in an iodixanol gradient, and transmission electron microscopy. Cell proliferation was determined by [^{3}H]-thymidine incorporation and cell migration assay was performed in a 48-well microchemotaxis chamber. Intracellular Ca^{2+} mobilization was determined with the fluorescent Ca^{2+} indicator fluo-3/AM, and AlexaFluor 488-conjugated phalloidine was used for imaging actin stress fiber formation.
Results: The purified TMVs settled at a density of ~1.110 g/mL, presenting membrane vesicles markers (CD63 and CD81), and almost all TMVs were spherical and bi-layered vesicles ranging from 40 to 150 nm in size. TMVs stimulated proliferation of tumor cells in a dose-dependent manner, with a maximum effect of 2.4 ± 0.2-fold increases upon exposure to 5 μg/ml of TMVs. TMVs increased migration of tumor cells in a dose-dependent manner, with a maximum effect of 4.1 ± 0.9-fold increases upon exposure to 1 μg/ml of TMVs. The treatment of 1 μg/ml of TMVs to fluo-3/AM-loaded tumor cells caused increased intracellular free Ca^{2+} release into the cytosol. Moreover, formation of actin stress fibers was enhanced by the treatment of 1 μg/ml of TMVs for 30 min. Theses results suggest that TMVs may deliver autocrine signals to target tumor cells.
Conclusion: We suggest that TMVs may be involved in promoting autocrine signaling to tumor themselves, neighboring or distant tumor cells for the rapid induction of proliferation, migration, as well as survival. Therefore, modulating biogenesis and functions of TMVs may be potential novel therapeutic strategies for treating pathological states including malignant tumors.

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Therapeutic intervention in a murine model of multiple myeloma with PEI nanocomplexes bearing an eIF5A siRNA and eIF5AK50R pDNA resulted in a significant anti-tumoural response

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The eukaryotic translation initiation factor 5A (eIF5A) is a highly conserved protein and the only known protein to contain the amino acid hypusine. Both EIF5A and the enzyme responsible for its post-translational modification, deoxyhypusine synthase (DHS), have been identified as critical factors for cell proliferation in yeast. Hypusine-modified eIF5A and DHS have also been identified as markers of neoplastic growth and metastasis, respectively. Recent studies have indicated that the unhypusinated form of eIF5A is a pro-apoptotic protein and appears to have a cellular function distinct from that of hypusine-modified eIF5A. Multiple myeloma (MM) is an incurable clonal plasma cell neoplasia and new therapeutic techniques are required for combating this disease. In this study, we have used a gene therapy approach to examine the effect of modulating hypusine-modified and unmodified eIF5A levels on the growth of human multiple myeloma subcutaneous tumours in mice. PEI nanoparticles were used to deliver plasmid DNA and siRNA to tumours in vivo using both intra-tumoral and intra-venous injection routes. An siRNA targeting human eIF5A was used to suppress levels of endogenous hypusinated eIF5A in tumours, while an RNAi-resistant plasmid expressing a mutant of eIF5A (eIF5AK50R), that is incapable of being hypusinated, was used to raise the levels of unmodified eIF5A in vivo. Intra-tumoural injection of PEI nanocomplexes containing eIF5A siRNA inhibited MM tumour growth by more than 80% (*** $p = 0.0003$) versus complexes containing a control siRNA, indicating that suppressing levels of hypusinated eIF5A has an anti-tumoural effect. PEI complexes containing an eIF5AK50R expression plasmid had a similar effect and inhibited tumour growth by more than 70% (** $p = 0.001$) versus complexes containing a control plasmid. Thus, MM tumour growth can be inhibited either by suppression of the growth-promoting hypusinated eIF5A or by increasing levels of the pro-apoptotic unhypusinated form of eIF5A. Intra-tumoural delivery of complexes containing both eIF5A siRNA and RNAi-resistant eIF5AK50R plasmid had a synergistic effect on tumour growth and resulted in significant tumour shrinkage, inhibiting tumour growth by 94% (*** $p = 0.0002$). Intra-venous delivery of eIF5A siRNA/eIF5AK50R PEI complexes also efficiently reduced tumour growth by 95% (** $p = 0.002$) indicating systemic delivery of the therapeutic is feasible. Preliminary in vitro investigations of siRNA-mediated suppression of eIF5A in a human multiple myeloma cell line suggest that siRNA treatment may sensitize cells to apoptosis through a reduction in NF-?B activation. Our findings indicate that both local and systemic delivery of eIF5A siRNA/eIF5AK50R pDNA PEI complexes result in a significant anti-tumoural response and may offer a new opportunity for therapeutic intervention in MM.

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Oncolytic adenovirus ONYX-411NTR enhances the antitumour efficacy of the bioreductive alkylator prodrug PR-104

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Background: PR-104A is a hypoxia-activated prodrug formed from the corresponding phosphate ester, PR-104, which is in phase I/II clinical trial (Clin Can Res 2007, 13: 3922). PR-104A is a dinitrobenzamide mustard (DNBM), known substrates for *E. coli nfsB* nitroreductase (NTR) (Can Gen Ther 2007, 14: 953). NTR can be expressed in tumours using the oncolytic adenovirus ONYX-411^{NTR} which replicates selectively in cells defective in the pRb pathway. Here we evaluate whether PR-104A is a substrate for NTR and whether PR-104 is suitable for combination with ONYX-411^{NTR} in a strategy known as virus-directed enzyme-prodrug therapy (VDEPT).
Methods: The IC_{50} for PR-104A was determined across a 5 cell line panel consisting of paired WT and NTR-expressing clones. Bystander cell kill by PR-104 was determined in the absence of virus by treating HCT 116 xenografts (nu/nu mice) comprising 25% stable NTR-expressing cells (75% WT). ONXY-411^{NTR} was delivered to HCT 116 tumours either systemically (single IV dose) or using a "pre-infection" model by inoculating 40 ONYX-411^{NTR} infected cells and 10^7 uninfected cells. Tumour viral titres were determined by plaque assay, NTR expression by western blot, intratumoural PR-104 metabolism by LC/MS/MS, and antitumour activity by tumour growth inhibition (TGI).
Results: Cell lines were on average 1600-fold more sensitive to PR-104A when engineered to express NTR. Intratumoural PR-104 metabolites redistributed efficiently as judged by 8/8 complete regressions in the 25%:75% NTR:WT tumours (day 24, TGI = 121%; $p < 0.001$). In contrast, 100% WT tumours failed to regress. Viral titres in the pre-infected tumours increased 2.8-fold ($p = 0.01$) following PR-104 treatment relative to virus only and was associated with significant antitumour activity (day 17, TGI = 94%; $p < 0.001$). Intravenous ONYX-411^{NTR} (5×10^8 pfu) seeded measurable tumour viral titre on day 3 (1.2×10^7 pfu/g) which amplified 24-fold by day 13 ($p < 0.01$) and correlated with increasing NTR expression. PR-104 treatment post-ONYX-411^{NTR} resulted in significant growth inhibition (day 26, TGI = 53%; $p = 0.007$) whilst PR-104 or ONYX-411^{NTR} alone were inactive. Concentrations of PR-104 activated metabolites were significantly higher in tumours treated with ONYX-411^{NTR} relative to untreated tumours suggesting that efficacy is due, at least in part, to enhanced PR-104 activation.
Conclusions: The efficacy of PR-104 is improved by expression of NTR in tumours following infection with ONYX-411^{NTR}.