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Treatment of Solid Cancers Using a New Cationic Cytolytic Peptide

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Background: Known for their bacteriotoxic effects, many cationic peptides also exhibit cytotoxic activity against cancer cells, mostly binding rapidly to the slightly negatively charged plasma membrane of cancer cells and disrupting it. In this work we have identified novel cationic peptide of 27 amino acids (YGRKKRRQRRRGKTLRVAKAIYKRYIE) that interacts rapidly with the plasma membrane of cancer cells, showing potential therapeutic efficacy against a number of cancer cell types, both *in vitro* and *in vivo*.
***In vitro* Experiments:** MTT and Live/Dead cytotoxicity assays on various human tumour cell lines (5 osteosarcoma, 6 glioma ,4 mammary carcinoma and 4 melanoma cell lines) showed up to 95% peptide induced cell death at concentration of 10−35 μg/ml of peptide, whereas normal human fibroblast and osteoblast cell lines were considerably less affected. Surface plasmon resonance (SPR) experiments revealed that the peptide binds strongly to negatively charged liposomes at neutral pH. Fluorescence spectroscopy demonstrated that the peptide induces significant membrane leakage of liposome contents. Time lapse scanning confocal microscopy on dsRED transfected tumour cell lines, showed dsRED leakage from the tumour cells within 1hr after treatment. Electron microscopy and time- lapse confocal microscopy confirmed these findings.
***In vivo* Experiments:** Pharmacokinetic profiling *in vivo*, using i.v injection of I^{125}-radiolabelled peptide, showed a peptide half- life *in vivo* of 1hr with ensuing renal clearance. 4T1 murine breast carcinomas were xenografted in 16 BALB/c mice and treated by a single-shot local bolus injection of 600μg/100μl of peptide. This resulted in up to 50% reduction of tumour size within 2−3 days post injection and reduced tumour re-growth in the following 4 weeks. Moreover, HF1GFPLuc human melanomas were xenografted in 30 NOD.CB17.Prkdcscid mice and then either treated by a single-shot local bolus injection of 1000μg/100μl of peptide or by weekly treatment. Tumour growth was monitored both by calliper measurement as well as by optical imaging. Again, a notable reduction in tumour growth was observed. Detailed tumour histology revealed large areas of necrosis and numerous pyknotic cells. Apoptotic cells were also detected by means of various immunohistochemical staining methods as well as by Tunel assay.
Conclusion: In sum, these findings indicate that the peptide both causes tumour cell death by membrane disruption and by apoptosis induction.

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Promising Anti-cancer Activity of a Novel Palladium (II) Complex on Human Breast Cancer Cells in Vitro and in Vivo

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Background: Treatment of breast cancer is still not satisfactory although new drugs have been introduced in recent years. Therefore, novel agents are required. Pd(II) complexes may be of importance for this aim.
Material and Methods: In this study, we synthesized ([Pd(sac)(terpy)](sac)-4H_2O) and tested its anti-cancer activities against human breast cancer cell lines, MCF-7 (estrogen receptor-positive) and MDA-MB-231 (estrogen receptor-negative). Anti-growth effect was assayed by the MTT and ATP assays, while the detection of programmed cell death (apoptosis) was performed by both caspase-cleaved cytokeratin 18 (M30-Antigen) and DNA-fragmentation assays *in vitro*. Apoptosis-related gene expressions were analyzed by RT-PCR and detection of protein expression levels by western blotting. Invasion capacity of cells was assayed by Matrigel® invasion assay. In addition, we investigated the anti-tumoral effect of the complex on Ehrlich ascites tumour (EAT) grown in female Balb-c mice *in vivo*.
Results: Results showed that the Pd (II) complex had a strong anti-growth effect on both cell lines in a time and dose dependent manner. IC_{50} values were 0.09 μM for MDA and 3.05 μM for MCF-7 cell line. The Pd (II) complex induced apoptosis at 3.12 μM in only MCF-7 cells. It was also effective in disrupting the formation of MDA-MB-231 tubules on matrigel, indicative of a putative anti-invasive activity. The gene expressions of cell death receptors of DR4 (TRAIL-R2) and DR5 (TRAIL-R1) were found to be induced by the complex. DR5 was also detected at the protein level. The anti-growth effect was confirmed by in vivo experiment in which the Pd (II) complex significantly inhibited the growth of the tumour.
Conclusions: The newly-synthesized Pd (II) complex has a strong anti-cancer activity against breast cancer cells by inducing apoptosis via cell death receptors *in vitro*. The complex is further able to significantly reduce the growth of tumour cells *in vivo* model. Taken together, the Pd (II) complex represents a potentially active novel drug for the breast cancer treatment.

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The New Synthetic Compound Pterocarpanquinone LQB-118 Induces Apoptosis in Acute Myeloid Leukemia Cells Through Survivin and XIAP Downregulation

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Background: The development for novel compounds exhibiting pro-apoptotic potential to target simultaneously a diversity of drug resistant mechanisms is highly desirable in acute myeloid leukemia (AML). The pterocarpanquinone-LQB-118 is a hybrid between pterocarpans and quinones, a synthetic compound structurally related to lapachol [2-hydroxy-3-(3-methyl-2-butenyl)-1.4-naphthoquinone]. We have previously shown that this compound was very effective in inducing apoptosis in cells from chronic myeloid leukemia. The major aim of the present study was to analyze the effect of this compound to observe whether LQB-118 would be equally effective in AML cells.
Materials and Methods: The cells viability (by MTT assay), the apoptosis index (by Annexin V on flow cytometry) and the caspase-3, caspase-9, survivin and XIAP expressions (by Western blot), were analyzed before and after *in vitro* LQB-118 treatment in Kasumi-1 AML cell line and cells from AML 17 patients. P-glycoprotein, p53, and Bcl-2 expressions analyzed by flow cytometry were correlated with the apoptosis index induced by LQB-118.
Results: LQB-118 6 μM reduced the cell viability of the Kasumi-1 cells by about 40% ($p < 0.05$) after 48 h and 72 h incubation. The reduction of the cell viability with LQB-118 9 μM was markedly higher (70%), when cells were incubated after 48 h and 72 h incubation ($p < 0.001$). Apoptosis was observed when Kasumi-1 cells were incubated with LQB-118 3 μM, and 9 μM after 24 h (30.7%, $p > 0.05$ and 85% $p < 0.01$), and after 48 h (54.2%, $p < 0.01$ and 92% $p < 0.001$), respectively. After 48 h incubation, LQB-118 caused an increase in caspase-3 activation as well in caspase-9 activation. LQB-118 3 μM was capable to induce a median of 28% and a median of 25% apoptosis after 24h and 48h incubations, respectively, in AML samples from patients exhibiting or not multifactorial multidrug resistance (MDR) phenotype.
Conclusions: LQB-118 was effective in triggering apoptosis in AML cells through the increased activity of caspase-3 and -9 and downregulation of survivin and XIAP in cells from patients presenting multifactorial MDR phenotype. Taken together, these data indicate LQB-118 as a promising candidate for clinical tests in AML patients.
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Carbon Nanovector Used as Transfectional Agent in NIH-3T3 Cell Mice and Potential RNAi Carrier for Solid Tumours

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Background: Cancer is a major public health concern worldwide. In Brazil, the estimates for the years 2010/2011 are approximately 489,270 new cases per year, being the second leading cause of death. Nowadays, chemotherapy, radiotherapy and surgery are used to treat cancer. Despite of being effective, some problems continue to occur, such as, antitumour agents nonspecific systemic distribution, low tolerance of some patients to treatment, high cytotoxicity to normal cells and multiple drug resistance development. It is extremely important that innovative technological methodologies can be designed to delineate tumour margins, separate cancer cells from normal, also identifying micrometastasis and if the tumour was completely removed. Within this perspective, many researchers in the