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PTEN target therapy for glioblastoma

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Background: Glioblastoma (GBM) is a highly lethal brain tumour present as one of two subtypes with distinct clinical histories and molecular profiles. The primary GBM subtype presents acutely as a high grade disease that typically harbors mutations in EGFR, PTEN and INK4A/ARF, and PTEN plays acritical role in a PI3 kinase/Akt signaling pathway. The potential role of the PTEN-PDZ binding domain in tumor suppression has been reported previously. Here, we synthesized a peptide consisted of PDZ binding domain in PETN conjugated with a protein transduction domain, and examined the potential of the peptide as a tumor suppressor agent targeting PTEN.
Material and Methods: Using a peptide synthesizer, we synthesized a peptide composed of PDZ binding domain in PTEN conjugated with TAT protein as a protein transduction domain. We used 4 glioma cell lines (YKG1, U251, U87, and T98) for this study and then examined PTEN/Akt expressions with western blotting and RT-PCR. The intracellular delivery of the peptide was evaluated using the fluorescence-labeling peptide. Growth inhibition, cell death, metabolic changes, and cell cycle inhibition of the synthesized peptide derived from PTEN morphological changes were examined with cell counting, soft agar colony formation assay, TUNEL assay, BrdU labeling assay, and immunocytohemistry.
Results: The synthesized peptide composed of PDZ binding domain in PTEN conjugated with TAT showed a significant growth inhibitory effect, cell cycle inhibition, induction of apoptosis in glioma cells at 1 to 10 μM in concentration in culture medium. PTEN/Akt expressions in glioma cells were correlated with growth inhibitory effects.
Conclusions: The intracellular delivery of PTEN-PDZ binding domain is a possible useful method as a glioma therapy when PTEN/Akt in cells does not function normally. The peptide composed of PTEN-PDZ binding domain conjugated with TAT is promising as a agent of a molecular target therapy for glioblastoma.

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Chemical proteomics profiling of erlotinib in NSCLC cell lines suggests novel mode of action

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Recently, it has been shown that NSCLC patients carrying an activating EGFR mutation in their tumour respond especially well to treatment with tyrosine kinase inhibitors like erlotinib and gefitinib (Paz-Ares et al. 2010). Whereas gefitinib does not seem to confer clinical benefit to an unselected patient population (Thatcher et al. Lancet 2005) erlotinib does (Shepherd et al. NEJM 2005; Cappuzzo et al 2010). In order to find out whether the differences seen in the clinical outcome can be attributed to differences in binding capacities of the two molecules to other enzymes than EGFR, a chemical proteomic study was carried out. Erlotinib was first chemically modified and conjugated to Sepharose in order to generate an affinity matrix. Non-small cell lung cancer cell lysates were pre-incubated with erlotinib before fractionation on the immobilized compound. Captured proteins were eluted, separated by SDS-PAGE and identified by nanoLC-MS/MS and protein database mining. Specific interactors were isolated by statistical analysis of the mass spectrometry signals. Besides EGFR, other enzymes were specifically displaced from the matrix by erlotinib. *In vitro* assays showed a differential inhibition of these enzymes between erlotinib and gefitinib. Currently the effect of erlotinib on selected cell lines in general (using e.g. expression profiling among other techniques) and on the downstream signaling of these proteins in particular is under investigation. In addition the cell lines are screened for mutations in the genes coding for these enzymes. Results of these experiments will be reported. In conclusion, this study may lead to the identification of additional tumor markers, which would allow better patient selection for treatment with erlotinib.

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Computational modeling and molecular optimization of stabilized alpha-helical peptides targeting NOTCH-CSL transcriptional complexes

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Aberrant transcription factor activity is a hallmark of cellular transformation and cancer progression. A notable example is found in the Notch signaling pathway, which is altered in more than 50% of T-cell acute lymphoblastic leukemia (T-ALL) cases as well as numerous other cancers through gain-of-function mutations in *NOTCH1*. Despite the obvious therapeutic potential of directly targeting transcription factor complexes, this class of proteins has been generally regarded as intractable or "undruggable." Employing a novel chemical approach that combined the surface recognition of a protein therapeutic with the synthetic accessibility of a small molecule, we previously reported the design, synthesis and characterization of a "hydrocarbon stapled" peptide inhibitor of the MAML1-NOTCH1-CSL transcription factor complex {Moellering et al., 2009, Nature, 462, 182−8}. As multiple NOTCH isoforms (NOTCH1−4) play non-redundant roles in an array of basal and pathogenic cellular processes, potent and specific inhibition of unique isoforms would be therapeutically desirable.
Toward this goal we have employed a complement of computational molecular modeling, structure-based design and iterative medicinal chemistry strategies to identify more potent and specific inhibitors of NOTCH-CSL complexes. Molecular dynamic MM-GBSA modeling was first used to quantify the contribution of individual MAML1 contact residues as a framework to select optimized binding interactions. The resulting model agreed with the conclusions of our original study and identified numerous hydrophobic residues present in SAHM1 that might be modified to optimize polar and Van der Waals contacts. With these positions in mind we designed a library of stapled peptide analogues with variable natural and non-natural amino acids at these positions. Compounds were assayed for biochemical inhibition of NOTCH complex formation by a novel nanobead-based competition assay in parallel with a NOTCH1-driven reporter gene assay to determine cellular efficacy. From this effort several optimized positions were found to confer increased biochemical and cell-based activity in analogue compounds. Homology modeling of individual NOTCH isoforms identified sites for differential targeting by SAHM peptides as well. Incorporation of optimized residues into larger SAHM analogues increased potency by more than one order of magnitude. Subsequent analysis of target gene repression, cell proliferation and pathway specificity in multiple cancer models supports the development of more potent and specific inhibitors of NOTCH function.

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Use of anti-VEGF therapy and antiestrogens in breast cancer cells: molecular mechanisms involved in response

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Background: Much is known about the mechanisms involved in the response to anti-hormonal treatment or those involved in the response to antiangiogenic therapy. It is also known the association between angiogenesis and hormonal status, both in physiological and pathological settings. However, the molecular and cellular mechanisms contributing to the efficacy of combined antiangiogenic-antihormonal therapy in breast cancer are still unknown. This combination is currently in clinical trials, but unfortunately there are scarce preclinical studies contributing to the rationale of combining antiangiogenic and antihormonal therapies.
Aims: To define the mechanisms involved in the response to combined antiangiogenic-antihormonal treatments in breast cancer cells.
Methods: Breast cancer cell lines with different estrogen dependence (MCF-7, BT-474, MDA-MB-231) were subjected to an estrogen gradient (estradiol), and treated with antiestrogens (fulvestrant or tamoxifen) plus bevacizumab (anti-angiogenic). Cellular proliferation and apoptosis were determined using the corresponding kits. Proliferation and survival intracellular signaling pathways, estrogen alpha and VEGF receptors activation and COX-2 expression were analyzed by western-blot using specific antibodies. VEGF-A concentration in culture medium was determined by ELISA.
Results: In estrogen-dependent breast cancer cells (MCF-7 and BT-474) the pro-proliferative effect of estradiol decreased after bevacizumab treatment (table 1).