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Chemical-genetic Screenings for Synthetic-lethal Interactions in Breast Cancer

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Background: Breast cancer (BC) has a huge epidemiological impact in developed countries and is a leading cause of cancer-related mortality among women. Despite progresses made in diagnosis and treatment, especially in its advanced or metastatic presentation, it is hardly curable. This is mostly due to the paucity of therapeutics that specifically target BC's genetic and phenotypic peculiarities. Synthetic-lethal therapeutics exploit cancer-associated mutations as Achilles' heels by taking advantage of the weaknesses that such mutations expose in malignant cells (i.e, PARP inhibitors for BRCA1/2-mutated cancers). Here, we report on the execution of chemical-genetic interaction screens aimed to identify compounds that specifically kill mammary epithelial cells engineered to express common BC-associated mutations.
Material and Methods: MCF10A, a spontaneously immortalized but non-transformed human mammary epithelial cell line, was engineered to express HRas, Her2/neu, PI3K H1047R or to stably silence the tumour suppressors PTEN, p53 and Rb. Cells engineered with empty retroviral vectors were used as controls. The introduced genetic modifications were verified by western blotting and by flow cytometry. The so-generated isogenic cells lines were probed against different collections of chemical compounds (including the Diversity and Mechanistic set from NIH's NCI Developmental Therapeutics Program) for a total of more than 10.000 genotype-compound combinations. Viability was assessed by colorimetric assay after a 48h-incubation. The experiments were performed in a 384-well format by means of a robotic liquid handling workstation.
Results: We developed a system to perform chemical-genetic screens in genetically-modified epithelial breast cells. Our approach is able to detect susceptibility phenotypes, since MCF10A cell engineered to overexpress HER2 do show a markedly increase susceptibility to Lapatinib, a dual EGFR/HER2 tirosine kinase inhibitor which is known to preferentially kill HER2-over-expressing cells. Moreover, previously reported resistance phenotypes due to aberrant activation of the PI3K-Akt and MAPK pathway were also readily detected in preliminary experiments.
Conclusions: We aim to identify synthetic-lethal compounds that may serve as leads for subsequent development of clinically-effective treatments for BC. The use of this type of chemical-genetic approach appears to be feasible and able to detect mutation-associated susceptibility phenotypes.

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Chemical Screening for Potentiators of Lapatinib Activity in Human Breast Cancer

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Background: 10−30% of breast cancers overexpress the oncogenic receptor tyrosine kinase HER2 as a consequence of gene amplification. In these tumours, HER2 acts as a driving oncogene which cancer cells are addicted to. Monoclonal antibodies against HER2 and small-molecule inhibitors of its tyrosine kinase activity proved effective in the treatment of HER2+ breast cancer. However, the benefit of these treatments is limited by primary and acquired resistance. Lapatinib is an inhibitor of the tyrosine kinase activity of both HER2 and EGFR and it is highly active against HER2-overexpressing breast cancers. Resistance to lapatinib in breast cancer is due to estrogen-dependent pro-survival mechanisms, and to activating mutations in the PI3K and ras pathways. We chose lapatinib as a drug model and performed chemical screens in order to identify compounds which synergistically enhance lapatinib efficacy in breast cancer cells.
Materials and Methods: The HER2+ overexpressing breast cancer cell line SKBR3 was used in our experiments. Cells were incubated in the presence or absence of lapatinib, and subsequently probed against the over 2000 compounds of the Mechanistic Set and the Diversity Set from NCI/NHI Developmental Therapeutic Program. Cells were plated in 384 well plates and 4 different concentration of each compound were tested. After 72h of incubation, cell viability was determined using a colorimetric assay and then the cooperative index (CI) was calculated as the sum of the specific cell deaths induced by the single agents divided by the specific cell death in response to their combination. CI values <1, indicate a synergistic effect between compounds.
Results: We identified 13 different compounds which showed a synergistic activity with lapatinib in SKBR3 cells. Most of these show antiproliferative activity in vitro in NCI-60 cell lines, but their molecular target is unknown. These compound were retested and 9 of them confirmed to synergistically potentiate the activity of the tyrosine kinase inhibitor. Follow-up experiments are now ongoing with the aim to define their mode of action and their usefulness in combination regimens.
Conclusions: Our preliminary results show that lapatinib activity can be potentiated in combination with compounds identified through chemical screens. Such drug combinations shall be further investigated and may lead to new treatment strategies for HER2-overexpressing malignancies.

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Enhanced Cellular Delivery of Idarubicin by Surface Modification of Propyl Starch Nanoparticles Employing Pteroic Acid Conjugated Polyvinyl Alcohol

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Background: Improved efficacy of anti-cancer agents with simultaneous reduction of their systemic exposure and non-specific toxicity is possible through enhanced internalization of nanoparticles encapsulating these. The routinely used hydrophilic stabilizers used in formulating nanoparticles may however hinder their interaction with the hydrophobic cell membranes. The present investigation attempts to overcome this problem by employing pteroic acid modified polyvinyl alcohol (ptPVA) as a novel surfactant to formulate idarbicin (IDA) encapsulated propyl starch nanoparticles [1]. This modification was hypothesized to enhance their uptake through improved adsorption of various proteins, the receptors of the latter being dominant on numerous cancer cells.
Materials and Methods: IDA-loaded ptPVA nanoparticles were formulated using solvent emulsification-diffusion technique and optimized for relative amounts of drug to polymer and stabilizer. The optimum formulation was characterized with regards to particle size, surface charge, morphology, drug encapsulation and loading and *in vitro* release pattern. Enhanced protein adsorption of ptPVA modified nanoparticles was confirmed employing BCA assay with BSA as the standard. The safety and efficacy of the nanoparticles was confirmed by MTT and ATPase cytotoxicity assays in Caco-2 cell line. Enhancement of cellular uptake was confirmed using Confocal laser scanning microscopy.
Results: A homogenous distribution of spherical IDA nanoparticles (245 nm) were formulated with IDA encapsulation of about 85%. These nanoparticles which exhibited a controlled drug release were found to be safe for cellular evaluations in both the cytotoxicity assays. Moreover an enhanced efficacy of the IDA nanoparticles was observed as compared to the free drug control thus indicating their efficacy. Higher protein binding was exhibited by ptPVA nanoparticles compared to non-modified ones indicating the possible influence of protein adsorption on improved internalization and hence efficacy. Furthermore, a higher cellular internalization was observed for ptPVA nanoparticles in HT-29 cells and A-549 cells, thus confirming the proposed hypothesis of higher protein adsorption being responsible for this effect.
Conclusion: Higher uptake and efficacy along with cellular safety indicate the potential of the IDA nanoparticles for *in vivo* evaluations to validate these results. Such selective internalization and efficacy in cancer cells is significant for toxic anti-cancer drugs like IDA.
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Docetaxel Delivery Mediated by Nanoparticles of Novel Hydrophobic Starch

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Background: Polymeric nanoparticles can overcome challenges in delivery of anti-cancer agents due to their enhanced internalization, retention and