Cytidine deaminase axis modulated by miR-484 differentially regulates cell proliferation and chemoresistance in breast cancer

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**Background:** Chemoresistance is a major clinical obstacle to achieve successful treatment in breast cancer. Cancer cells construct a complicated regulatory network to develop resistance in response to chemotherapy; however, beyond chemoresistance, there has been little study of how the evolution of chemoresistance in cancer affects other aspects of disease pathogenesis.

**Materials and methods:** We identified a novel chemoresistance-associated axis, in which cytidine deaminase (CDA) is a direct downstream target of miR-484 via an integrated mRNA-miRNA network, using a gemcitabine-resistant cell model. And we explored the biological characteristics and clinical signature of this miR-484/CDA axis in breast cancer.

**Results:** We show that an important chemoresistance axis driven by CDA also acts to suppress cell cycle progression by regulating cyclin E-CDK2 signaling. We found CDA to be regulated by miR-484 in a gemcitabine-resistant model of breast cancer. Elevating miR-484 expression reversed CDA effects, thereby enhancing gemcitabine sensitivity, accelerating cell proliferation and redistributing cell cycle progression. Conversely, elevating CDA to restore its expression counteracted the chemosensitization and cell proliferative effects of miR-484. In clinical specimens of breast cancer, CDA expression was frequently downregulated and inversely correlated with miR-484 expression. Moreover, high expression of CDA was associated with prolonged disease-free survival in breast cancer patients without gemcitabine-based treatment.

**Conclusion:** Our findings established that miR-484-modulated CDA has a dual impact in promoting chemoresistance and suppressing cell proliferation in breast cancer, illustrating the pathogenic tradeoffs associated with evolution of chemoresistance in breast cancer treatment.
Multidrug resistance revisited: A new mechanism and a possible solution

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More than four decades have passed since ABC transporters were identified as the culprit of multidrug resistance (MDR). Extensive studies led to development of highly effective inhibitors of the ABC transporters, but so far these inhibitors failed in clinical trials. We explored the possible existence of MDR mechanisms other than drug transport. Major chemotherapeutic agents, including doxorubicin, 5-FU, vincristine and bortezomib trigger extensive endoplasmic reticulum (ER) stress. We observed that MDR tumor cells are also resistant to ER stress-triggered cell death, suggesting that ER stress is an important mechanism by which chemotherapy kills tumor cells. The transcription factor C/EBP beta is over-expressed in many tumor types, naturally appearing in two isoforms: a full size active form termed LAP, and a dominant negative truncated form termed LIP. Previously, we demonstrated that LAP augments tumor progression by attenuating ER stress-triggered cell death, whereas LIP attenuates tumor progression by enhancing ER stress-triggered cell death (2). We now find that a broad range of MDR cell lines, as well as primary MDR tumor cells, lack LIP. Importantly, restoring LIP abolished the chemoresistance in all of these MDR cell types. On further analysis, we discovered that LIP expression was identical in chemosensitive cells and in their drug-selected MDR subclones. However, LIP was rapidly degraded by proteasomal and lysosomal proteases in the MDR cells and not in the chemosensitive cells. We then demonstrated that proteasome and lysosome inhibitors attenuated LIP degradation in MDR cells, rendering them more sensitive to chemotherapy. A combination of such inhibitors, currently used in the treatment of myeloma (bortezomib, carfilozumib), and malaria (chloroquine), should be explored as potential means for reversing MDR.

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Rapid induction of doxorubicin resistance in connected microenvironment elucidates novel molecular mechanisms

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Introduction: Emergence of drug resistance is the most critical cause of failure in cancer treatment. Developing alternative medicine usually requires good grasp of molecular mechanisms, which is hampered by difficulties in obtaining drug-resistant cells.

Materials and method: Here we introduce a microfluidic device based on nanochip technology that consists of approximately 500 hexagonal micro-compartments of continuous concentration gradient. Genetic mutations and expression alterations leading to drug resistance were investigated by whole exome and transcriptome sequencing, respectively.

Results and discussion: We verified that U-87 glioblastoma cells cultured on the nanochip under doxorubicin media showed drug resistance as early as seven days. Subsequent exome sequencing has identified 61 candidate mutations. Gene ontology terms for molecular function were statistically enriched in ‘nucleoside binding’ and ‘ATP-dependent helicase activity’. This is in excellent agreement with the previous knowledge that doxorubicin inhibits the enzyme topoisomerase II by intercalating between two base pairs of the DNA double helix. From the mutation and expression data, we were able to identify three mechanisms of resistance development to doxorubicin. Firstly, FLNA (Filamin A) that was known to regulate the influx and efflux of topoisomerase II poisons was mutated by a frameshift insertion. Secondly, mutation of three genes (NLRP13, NSD1, CARD6) and overexpression of inflammatory cytokines (CCL2, CXCL1, CXCL2, IL6, IL8, IL1B, TNF) indicate independently the role of NF-kB via the NOD-like receptor signaling pathway. Lastly, overexpression of aldo-keto reductase enzymes, converting doxorubicin into doxorubicinol, is likely to contribute to resistance development as well. Consequences of loss-of-function variants were verified by siRNA knockdown experiments.

Conclusion: In this work, we have demonstrated that a microfluidic device of concentration gradient induces cancer drug resistance rapidly in vitro and that subsequent analysis of mutation and expression data reveals the molecular mechanisms of resistance development successfully. This combination of nanochip and deep sequencing technologies would provide a promising platform for overcoming cancer drug resistance.
From personalised medicine to personalised chemoresistance mechanisms: effective targeting of molecular subtypes of pancreatic cancer

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Pancreatic cancer (PC) is the fourth leading cause of cancer death with few effective therapies. Our increasing appreciation of the genomic heterogeneity of cancer suggests that the failure of definitive clinical trials to demonstrate efficacy in the majority of cases is likely due to the low proportion of responsive phenotypes. As a consequence, novel strategies to approach this disease are required. Here we show that through a combined bench-to-bedside, and bedside-to-bench strategy using appropriate well-characterised model systems we can identify molecular phenotypes with significant responses to specific therapies (1). We identify candidate molecular phenotypes using a combination of assays including global analysis of gene expression, copy number alteration, structural variation and mutation using next-generation sequencing (2 3). Interestingly, high frequency structural variation seen on whole genome sequencing, is associated with mutations in many genes involved in DNA damage repair and importantly, confers responses to DNA damaging agents. This molecular signature is observed in ~15% of primary operable pancreatic cancers. Finally, we show how patient-derived xenografts present effective tools to examine chemoresistance mechanisms in PC and ways to test rationally designed treatment combinations. Ultimately, we are building a knowledge bank of responsive phenotypes to inform clinical decision-making, creating opportunities for the rescuing and repurposing of existing therapeutics, and the development of novel therapeutic strategies.

References:
Selective transfer and inhibition of multidrug resistance in cancer cells

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Introduction: Multidrug resistance (MDR) is a form of resistance that results from the overexpression of drug efflux transporters, P-glycoprotein (P-gp) or multidrug resistance protein-1 (MRP-1). The overexpression of P-gp by cancer cells is a major obstacle to the successful treatment as it prevents the intracellular anticancer drug uptake by virtue of its drug efflux capacity. We have confirmed the intercellular transfer of P-gp via extracellular vesicles known as microparticles (MPs) and proposed the presence of an 'interaction complex' in mediating this. Similarly, we have also shown that MPs shed from drug-resistant breast cancer cells (Dx) transferred P-gp selectively to malignant breast cancer cells whereas MPs derived from drug-resistant leukaemia cells (VLB100) transfer P-gp to malignant and non-malignant cells.

Aims: 1) Define the protein signature that is essential in governing the selective tissue transfer of P-gp in recipient cells by resistant breast cancer cells.
2) Individually validate the role of the constituents comprising the interaction complex.

Materials and Methods: MPs were isolated from Dx and VLB100 cells. Isolation of MPs, protein extraction, 1D-SDS-PAGE fractionation, in-gel trypsin digestion, nano LC/MS/MS and data analysis were performed as previously described [1]. Similarly, we sequentially silenced the group of proteins and observed the drug efflux capacity of cancer cells.

Results and Conclusion: We identified 40 unique proteins in Dx-MPs that are candidates for governing the tissue selective transfer of P-gp by these MPs. Of these proteins CD44, CD73, CD9, CD13, amongst others have been consistently shown by us to be key candidates in this process [1]. We found the higher accumulation of anticancer drugs in the cancer cells after disrupting the targeted interaction complex. We demonstrate the implication of the presence of these proteins in the regulatory control of P-gp and identify them as a potential target in clinical oncology.

Reference
Family with sequence similarity 60a (FAM60a) regulates drug resistance of A549 lung adenocarcinoma cells to cisplatin

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Background: Cisplatin is a widely used chemotherapeutic drug in lung cancer management. However, the majority of cancer patients will eventually show resistance to cisplatin. In this study, we aimed to investigate the potential mechanisms underlying cisplatin resistance in lung cancer.

Materials and methods: In our preliminary work with genes knockdown, si-FAM60a was found to be involved in the sensibilization of lung adenocarcinoma A549 cells to cisplatin by high content screening. On this basis, we further investigated the potential mechanism of FAM60a in cisplatin resistance in A549 cells by real time-PCR, western blotting, flow cytometry, gene over-expression technology, and ChIP assay. The animal model of human subdermal xenograft A549 cells into BALB/c nude mouse was used to test the influence of FAM60A overexpression on tumor growth to cisplatin in vivo.

Results: The mRNA and protein of FAM60a were significantly increased in A549 cells with high cisplatin treatment. Over-expression of FAM60a promoted the expression of resistance-related proteins (MDR and P-gp), inhibited the expression of apoptosis-related proteins (caspase 3 and caspase 8), and prevented the apoptosis of A549 cells, especially under cisplatin stimulation. The expression of SKP2 mRNA was down-regulated in the FAM60a siRNA A549 cells, whereas over-expression of FAM60a up-regulated the expression of SKP2 mRNA. Meanwhile, si-SKP2 inhibited the expression of resistance-related proteins, increased the expression of apoptosis-related proteins, and promoted the apoptosis of A549 cells, especially under cisplatin stimulation. The volume of xenograft tumor was larger in the FAM60a overexpression group, while si-SKP2 could restrict its resistance to cisplatin in vivo. Moreover, the regulation between FAM60a and SKP2 was found to be mediated by the decreased histone acetylation of CUTL1.

Conclusions: Our results suggest that FAM60a leads to up-regulation of SKP2 through CUTL1 acetylation, enhancing cell resistance to cisplatin in A549 cells, which may provide an attractive therapeutic target to modulate drug resistance in lung cancer.

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FAM60a overexpression increased the xenograft tumor growth under cisplatin treating, which could be restricted by si-SKP2 in vivo
Hypoxia Inducible Factor-1α determines the sensitivity to aromatase inhibitor (AI) in ER-positive breast carcinoma

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Purpose: Endocrine resistance is a major obstacle limiting the clinical benefit of endocrine therapy in breast cancers and its mechanism is still not fully understood. The present study aimed to investigate the role of hypoxia inducible factor-1α (HIF-1α) in endocrine resistance of estrogen receptor (ER)-positive human breast cancer.

Experimental design: Paired breast cancer specimens from both baseline core needle biopsy and post-treatment surgery or follow-up core needle biopsy after primary endocrine therapy were collected from each patient for immunohistochemistry (IHC) testing. The level of HIF-1α was assayed in 52 primary breast cancer patients who received neoadjuvant endocrine therapy for at least 3 months by immunohistochemistry staining before and after administrating letrozole, an oral non-steroidal aromatase inhibitor for the treatment of hormonally-responsive breast cancer. The expression levels of HIF-1α were compared between pre-treatment and post-treatment samples by using the Wilcoxon test. Additionally, a HIF-1α stably expressing cell line was established (MCF-7/HIF-1) and was injected subcutaneously into the flank of each mouse. Mice were randomly treated with the anti-estrogen agent fulvestrant. Tumor xenografts were measured with calipers twice a week. A P value of less than 0.05 was regarded as statistically significant.

Results: In our clinical case series analysis, the expression levels of HIF-1α protein at baseline were negatively associated with clinical outcome (P<0.001, Chi-square test, Fig. 1A). A significant increase in the levels of HIF-1α protein was shown in post-treatment residual tumors (indicating insensitivity to endocrine therapy) compared with that in the baseline biopsy samples (Fig. 1B), which were irrespective of the efficacy of primary endocrine treatment (overall score or intensity score, P<0.0001 or P=0.0002, respectively; Paired t test) (Fig. 1C, D). In animal studies, xenografts stably expressing HIF-1α were resistant to endocrine therapy with fulvestrant compared with the effects in control xenografts.

Conclusions: HIF-1α may be a crucial determinant of resistance to endocrine therapy and should be considered as a future therapeutic target for overcoming endocrine resistance in ER-positive breast cancer.

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Phase I study of the DNA repair inhibitor DT01 in combination with radiotherapy

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Background: DT01 is a double stranded DNA oligonucleotide conjugated to cholesterol, which mimics "false" double strand breaks (DSB). DT01 triggers DNA repair proteins trapping, thereby inhibiting their repair activity. In preclinical models, DT01 displayed antitumor activity as a single agent and in combination with radiotherapy (RT) in several tumor types including melanoma without additive toxicity. DT01 addressing and mechanism of action seems specific of tumor cells sparing healthy tissues.

Methods: Intratumoral and/or subcutaneous peritumoral injections of DT01 were evaluated in combination with RT in a first-in-human phase I trial in patients with unresectable skin metastases from melanoma. Each patient received RT (3Gy/d, 5 days/week for 2 weeks) on all selected tumor lesions, while only one or two lesions were treated with DT01 three times a week during both weeks of RT. A 3+3 dose escalation design was used. DT01 dose levels explored were 16, 32, 48, 64, and 96 mg with an expansion cohort at 96 mg. Tolerance was assessed using NCI CTCAE v4 and efficacy using modified RECIST criteria 1.1.

Findings: Twenty three patients were included in the trial. All of them were evaluable for safety and pharmacokinetics. Twenty one patients were evaluable for efficacy, corresponding to 76 irradiated lesions of which 41 were injected with DT01. As no dose-limiting toxicity was observed, the maximum tolerated dose was not reached. Most frequent adverse events were reversible grade 1 and 2 injection site reactions. Pharmacokinetics analyses detected a systemic passage of DT01. An objective response was observed in 51 lesions (67%), including 23 complete responses (30%). Overall response rate correlated with DT01 systemic exposure in the subgroup of lesions that were not injected with DT01, suggesting a systemic effect of DT01.

Interpretation: DT01 in combination with RT is safe and provides antitumor activity in patients with skin metastases of melanoma. The systemic passage of DT01 and the correlation between DT01 systemic blood exposure and efficacy suggests a bystandersystemic effect of DT01.
Targeting STAT3 with an oral small-molecule inhibitor to reverse chemotherapy and radiation resistance

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Introduction: STAT3 has been validated as a target for treatment of many cancers, including ER-, PR- and HER2- (triple-negative) breast cancer (TNBC) and head and neck squamous cell carcinoma (HNSCC), especially to eliminate cancer stem cells, which are linked to chemotherapy and radiation resistance in these and other cancers. Yet, a STAT3 inhibitor has not entered the clinic.

Materials, methods & results: C188, a small-molecule STAT3 inhibitor previously identified by us (1), was used in a hit-to-lead program to identify C188-9. C188-9 binds STAT3 with high affinity (4.7 ± 0.4 nM) and is a marked improvement over C188 in its ability to inhibit STAT3 binding to its pY-peptide ligand and to inhibit cytokine-stimulated pY-STAT3 (2-4). We examined C188-9 alone and in combination with docetaxel for anti-tumor effect in two pY-STAT3-positive, docetaxel-resistant TNBC PDX models we developed - 4195 and 4272 (5). Using RECIST criteria, C188-9 converted PDX 4195 from docetaxel- resistant to docetaxel-sensitive. While not reversing docetaxel resistance in PDX 4272, addition of C188-9 to docetaxel prevented tumor growth vs. docetaxel alone (p<0.05). Importantly, C188-9 reduced pY-STAT3 levels in PDX tumors harvested 24 hours after the last treatment (p<0.05). C188-9 also reduced constitutive pY-STAT3 levels in UM-SCC-17B (a radioresistant HNSCC cell line) and inhibited anchorage dependent and independent growth of these cells. In addition, treatment of nude mice bearing UM-SCC-17B xenografts with C188-9 prevented tumor xenograft growth and modulated many STAT3-regulated genes involved in oncogenesis and radioresistance, as well as radioresistance genes regulated by STAT1. C188-9 demonstrated excellent oral bioavailability, was well tolerated by mice, rats, and dogs, and was concentrated 3-fold in tumors.

Conclusions: Thus, C188-9 shows promise in reversing chemotherapy resistance in patients with pY-STAT3-positive TNBC, as well as radiation resistance in patients with pY-STAT3- and/or pY-STAT1-positive HNSCC.

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Bufalin suppresses cancer stem like-cells in gemcitabine-resistant pancreatic, cancer cell line

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Cancer stem cells (CSCs) play an important role in cancer, as these cells possess enhanced tumor-forming capabilities and are resistant to current anticancer therapies. Agents that can suppress CSCs would provide new opportunities to fight against tumor proliferation and metastasis. In the present study, we aimed to evaluate the effect of bufalin on pancreatic CSCs in vivo and in vitro. Using serum-free suspension culture, we enriched tumor spheres in gemcitabine-resistant human pancreatic cancer cell line which has a higher percentage of CSCs and demonstrated that these sphere cells had characteristics of CSCs. Using this cancer stem like-cells as a model, we tested the effect of bufalin on the pancreatic CSCs. Our results demonstrated bufalin reduced the number of tumor spheres along with downregulation of CD24 and ESA. Furthermore, in a subcutaneous xenograft model of implanted gemcitabine-resistant MiaPaCa2 (MiaPaCa2/GEM), bufalin inhibited tumor growth and prolonged the time of tumor formation. Additionally, the expression of CD44 and ESA were also inhibited in bufalin-treated mice. Interestingly, in another cancer model with tumor cells injected via the tail vein, fewer metastasis lesions were detected in the bufalin group.

Taken together, our results suggest that bufalin suppresses pancreatic CSCs in MiaPaCa2/GEM
Gold nanoparticles conjugated with rituximab lower the chemoresistance of chronic lymphocytic leukemia

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Background: Chronic lymphocytic leukemia (CLL) is a monoclonal disorder that is characterized by a continuous accumulation of malignant lymphocytes. CLL is the most common form of leukemia found in the Western world. Gold nanoparticles are extensively exploited in biomedical applications because of their easy preparation, ready bioconjugation and potential biocompatibility.

Aims: The aim of the current study was to evaluate in vitro the anti-tumor efficacy of gold nanoparticles (GNPs) conjugated with the monoclonal antibody-based drug rituximab for the treatment of chronic lymphocytic leukemia. This approach based on gold proposes a novel platform therapy with minimal toxicity and increased efficacy profiles for the destruction of hepatic cancer cells.

Methods: GNPs, stabilized with a monolayer of L-aspartate and additional cytostatic drugs, were successfully used as a complex tumor-targeting drug-delivery system. The drug was non-covalently conjugated onto the hydrophilic assemblies of GNPs-L-aspartate nanostructure. Transmission electron microscopy was used to characterize the morphological and structural properties of these drug-metallic nanostructures. The other tests included UV-Vis-NIR absorption spectroscopy, dynamic light scattering, zeta potential, fluorescence and/or surface enhanced Raman scattering (SERS) and found the GNPs to be biochemically stable and detectable inside cells. The functional tests included MTT assay, cell counting, cell cycle analysis and apoptosis assay.

Results: The cellular proliferation rates in the presence of rituximab delivered by the GNPs were found to be statistically lower than those of cells exposed to the cytostatic drugs alone, indicating that GNPs facilitated an increased susceptibility of cancer cells to this new drug.

Conclusion: This approach could offer a new chemotherapy strategy for patients diagnosed with chemotherapy-resistant CLL.