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Antibody-independent enrichment of live circulating tumor cells (CTCs) from a variety of cancer types

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Background: Circulating tumor cells (CTCs) are used clinically as biomarkers for monitoring metastatic disease progression. However, CTC capture has been hampered in various cancer types mainly due to the limitation of antibody capture methods, primarily based on EpCAM. We developed a new CTC enrichment device, ApoStream™ that utilizes differences in cell morphology between normal cells and cancer cells. The device is based on dielectrophoretic field-flow fractionation in a continuous flow microfluidic chamber to separate CTCs from other healthy blood cells.
Materials and Methods: To demonstrate the performance of the ApoStream™ device, cancer cells were spiked into peripheral blood mononuclear cells (PBMCs) from normal donor blood and isolated based on their morphological differences. Cancer cell recovery, viability, immunofluorescence detection and FISH were demonstrated on cells isolated from the ApoStream™ device from lung, prostate and melanoma cancers. Lung cancer blood samples were compared in a paired sample study with CellSearch®. Cells isolated from ApoStream™ were stained for cytokeratin (CK+)/CD45-/DAPI+ and melanoma CTCs using S100+, and imaged using laser scanning cytometry (LSC). CTC morphology was also confirmed with H&E staining.
Results: ApoStream™ yielded a recovery of 67±15% with more than 1000 times enrichment from samples spiked with ovarian cancer cells (SKOV3). The viability of cells after ApoStream™ separation was more than 90% and isolated cells were grown successfully in culture. High CTC recovery from lung (n = 27), prostate (n = 10) and melanoma (n = 7) cancer patient blood samples was achieved with counts ranging up to 3500 CTCs per 7.5 ml blood. Further, there were no false-positive CTCs from normal donor blood controls demonstrating ApoStream™'s specificity. When compared to the CellSearch® system, ApoStream™ isolated a significantly higher number of CTCs from lung cancer patient blood samples (range:0–1213, mean:259 versus range:0–340, mean:14) showing the effectiveness of ApoStream™ in isolating EpCAM-negative CTCs. In addition, FISH analysis was successfully performed on ApoStream™ enriched cells.
Conclusion: ApoStream™ technology permits antibody-independent enrichment of CTCs from various types of cancers with high recovery for further downstream characterization including protein, RNA and DNA analysis. Viable CTCs enriched by 'ApoStream™' allow for culturing of these rare cells for drug screening experiments and ultimately implementation for personalized cancer therapy.

PP 26

IGF-1R signaling in ER+ and triple negative breast cancer − a potential target for therapy?

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Background: IGF1 and its signaling network control growth, development and regulate proliferation and survival. IGF-1R is overexpressed in many types of solid tumors including ER+ and triple negative breast tumors. There appears to be a crosstalk between the IGF1R system and ER signaling in ER+ breast cancer. Indeed, there is in vitro evidence showing that increased signaling through IGF1R confers resistance to ER targeting agents. Consequently, inhibitors of the IGF1R are in active development. Since IGF-1R is involved in the progression of breast cancer and resistance to systemic (including endocrine) treatment; suppression of this pathway by administration of IGF-1R mAB may therefore be efficacious in ER+ and TNBC models.
Materials and Methods: To examine this possibility, BIIB022 (a recombinant human AB directed against IGF-1R) was treated in ER+ (MCF7 & T47D) and TNBC cells (HCC1937 & MDA-MB-231).
Results: Treatment of ER+ and TNBC cell lines with BIIB022 in vitro showed: (1) 15−25% cell killing and 50−60% anti-proliferative activities by MTT and 3D-ON TOP colony formation assay respectively, (2) dose- and time-dependently blocked p-AKT (both at Ser473 and Thr308), (3) blocked activation of P70S6K in ER+ cells, but not in TNBC cell lines, (4) both IGF1 and estrogen-induced p-AKT and pP70S6K were abrogated by prior treatment of BIIB022 in ER+ cells, (5) IGF1-induced AKT phosphorylation was inhibited, but phosphorylation of downstream effectors of mTOR (p70S6K or S6K Ribosomal Protein) was not inhibited in TNBC cells by prior treatment of BIIB022, (6) inhibition of IGF-1R by BIIB022 lead to activated ERK both in ER+ and TNBC cells, (7) BIIB022 significantly reduced HIF1α expression following IGF1 stimulation in ER+ cells, and (8) integrin-dependent breast tumor cell migration was significantly abrogated with BIIB022 in association with RAC1-GTP inhibition.
Conclusion: Our preclinical in vitro data demonstrate (1) IGF-1R signaling cascade is not completely inhibited by prior treatment of BIIB022, (2) Blocking IGF-1R signaling inhibits estrogen-mediated AKT-mTOR pathway activation (non-genomic function of ER), (3) From this study, we can suggest that the RAS-MAPK or mTOR kinase inhibitor must be used in conjunction with IGF-1R inhibition for maximum anti-tumor effect in both ER+ and TNBC models.

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Pleiotropic influences of radio- and chemotherapy on auto-antibodies warrant caution for their use as biomarkers of tumor response: the anti-GRP78 paradigmatic example

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Background: Radio- and chemotherapy are common anticancer strategies applied nowadays to treat cancer patients. Although biomarkers are developed to predict and track tumor response, pleiotropic effects of anticancer treatments on global patient biology, and thus on the relevance of observed changes in biomarkers levels, are largely underestimated. In particular, biomarkers based on the immune response could be misleading considering the myelotoxicity of chemotherapy and the local pro-inflammatory effects of ionizing radiations. Here, we examined how an emerging class of seric cancer biomarkers, namely auto-antibodies (AAbs) directed against tumor-associated antigens (TAAs), may be influenced by conventional anticancer modalities.
Materials and Methods: We first performed serological proteome analysis (SERPA) as an integrated proteomic workflow to identify TAAs in mice bearing syngeneic lung carcinoma. We then applied radiotherapy (16 Gy) and chemotherapy (100 mg/kg cyclophosphamide) on tumor-bearing mice and studied the consequences on AAbs production.
Results: We first identified different protein candidates showing a specific immune response against the serum of tumor-bearing mice and selected GRP78 (Glucose-Regulated Protein 78) for further evaluation. Although we used irradiation and cyclophosphamide regimens that inhibited tumor growth to the same extent, we found that chemotherapy was associated with a reduction in the serum titer of anti-GRP78 AAbs ($p < 0.02$, $n = 10$), whereas radiotherapy increased it ($p < 0.001$, $n = 15$). We next documented that the decrease in anti-GRP78 AAbs (and total circulating IgG) after cyclophosphamide administration was related to a net reduction in the population of antibody-producing B-cells. Local tumor irradiation did not influence the number of B cells even though it led to an overall decrease in circulating antibodies due to their massive trapping in irradiated tumors. This phenomenon however did not preclude the post-radiation increase in circulating GRP78 AAbs concentration that we could attribute to a local increase in GRP78 protein in the irradiated tumors.
Conclusion: In this study, using anti-GRP78 antibodies a paradigmatic immune-based marker of tumor growth, we showed that conventional anticancer therapies may dramatically influence the titer of AAbs for other reasons than a direct tumor response. This should draw the attention of investigators towards the therapeutic status of cancer patients recruited in studies aiming to identify new AAbs as cancer biomarkers.

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Clinical and genetic characterization of dihydropyrimidine dehydrogenase deficiency in fluoropyrimidine-treated patients carrying the DPYD*2A allele

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Background: The therapeutic efficacy and toxicity of fluoropyrimidines are, at least in part, related to the balance between anabolism of the drug to its nucleotides, which inhibit thymidylate synthase and are incorporated into RNA and DNA, and the catabolic pathway dependent on dihydropyrimidine dehydrogenase (DPD), which is the initial and rate-limiting step in pyrimidine degradation. Over the last decade, it has become clear that DPD regulates the amount of 5-FU available for anabolism thereby affecting its pharmacokinetics, toxicity, and efficacy. Moreover, an uncommon variant of the DPD gene, consisting of a G to A mutation in the splicing recognition sequence of intron 14 (IVS14+1G>A) of the DPD-encoding gene (DPYD*2A), produces a non-functional enzyme due to skipping of exon 14 and is potentially associated with life-threatening toxicity.
Materials and Methods: Six patients given FOLFOX, capecitabine or 5-FU test dose (425 mg/mq) were genotyped. They suffered from the following toxicities (WHO criteria): diarrhea and febrile neutropenia grade 3−4, nausea-vomiting, stomatitis, piastrinopenia, alopecia, hand-foot syndrome grade 3 and anemia grade 2. Blood samples for DNA analysis were collected and used to screen patients for DPD polymorphisms by PCR and automatic sequencing of the entire coding region.