Metabogenomics in cancer

Integrative and comparative analyses of multiple transcriptomics, proteomics and metabolomics datasets require an intensive knowledge of tools and background concepts [1]. Systems biology is the sum of knowledge from novel methodological approaches (including omics), and the further translation of this knowledge into an understanding allowing for prediction of the behavior of the complex and dynamic networks that regulate living specimen. There is currently no definition of the term metabogenomics, but in the context of this lecture I will define it to embrace approaches seeking to reveal associations and interactions between the genomic/transcriptomic and metabolomic levels in a more simplified form than a pure systems biology approach would do. A challenge in this field is that a direct association between gene expression data and metabolite concentration should not always be expected. Enzymatic activity does not necessarily correlate directly with gene expression levels. Post-translational modifications and feedback mechanisms regulate the metabolite concentrations, keeping them within restricted limits. Furthermore, metabolite levels reflect the net activity of several enzymes.

The lecture will cover several areas within such work. Starting with the importance of methods for collecting representative and high quality tissues or cells suited for such analyses [2]. The lecture will focus on studies where metabolite analyses have been performed by high resolution MR spectroscopy (HR MRS), including analyses of tissue using HR magic angle spinning (MAS) MRS[3] and *in vivo* MRS. HR MAS gives the opportunity to obtain metabolic profiles from intact tissue, enabling subsequent analysis on the genetic level from the same samples. A metabogenomic approach would typically be interesting in studies concerning the points below, and the lecture will cover examples from research within these areas:

1) Better mechanistic understanding of why certain metabolic profiles are observed
2) Multilevel stratification of patients in risk groups or to treatment
3) Detection of biomarkers for response to traditional or targeted therapy and detection of new treatment targets for drugs
4) Understanding functional consequences of mutations

Understanding the metabolic profile

Altered metabolism is an emerging hallmark of cancer [4]. The cells must adapt to a hostile microenvironment with restricted supply of nutrients and they need to convert nutrients into biomass while maintaining sufficient energy production [5]. The cancer-specific metabolic phenotype with increased glucose consumption and aerobic lactate production is well known. In addition, abnormal phospholipid metabolism and shunting of metabolites from glycolysis into the pentose phosphate pathway is also commonly associated with cancer [5,6]. Dysregulated choline metabolism is a well-known feature of breast cancer [7], but the underlying mechanisms are not yet fully understood. Patient-derived xenograft (PDX) models are considered to represent the heterogeneity of human cancers, and can be useful for further understanding of the choline metabolism. The metabolomic and transcriptomic characteristics of a large panel of breast cancer PDX models have been mapped with focus on choline metabolism. The majority of the PDX models were classified as the genetic subtypes basal-like and

luminal B (poor and good prognosis, respectively) and were associated with significantly different choline metabolic and gene expression profiles [8]. One of the interesting features was the elevated ratio of phosphocholine(PCho)/ glycerophosphocholine (GPC) found in luminal B compared to the basal like xenografts [8,9]. This is not in correspondence to results from *in vitro* studies of breast cancer, suggesting that microenvironmental factors play a role in the *in vivo* regulation of choline metabolism. In prostate cancer, low concentrations of citrate and high concentrations of choline-containing compounds (ChoCC) are typically observed [10,11]. A better understanding of the underlying mechanisms could both enhance the understanding of the malignant progression, and reveal targets for novel therapeutics. When combining data from histopathology, gene expression analysis and metabolic profiling, distinct gene products were found to predict the reduced citrate concentration and accompany the increased ChoCC [12].

Multilevel stratification of patients in risk groups or to treatment

Optimal stratification of breast cancer patients is still a major challenge in oncology. Based on gene expression profiles, five molecular subtypes of breast cancer, associated with differences in clinical outcome, have been classified [13,14]. Similarly, it has been shown that patients can be grouped into metabolic subtypes with different clinical outcome [15,16]. The clinical implementation of the molecular subtypes in breast cancer is still limited, and even within the subtypes with good prognosis (i.e. Luminal A), some patients experience early relapse. Metabolic markers that could further subgroup the intrinsic gene subtypes are therefor of interest. It has been shown that a combination of transcriptomics and metabolomics can refine the subclassification of breast cancer as well as reveal relationships between these molecular levels [17].

Detection of biomarkers for response to traditional or targeted therapy in genetic subtypes and detection of new treatment targets for drugs

The aberrant phospholipid metabolism in cancer is a potential target for treatment [18]. Especially the high choline kinase-α (Chk-α) expression frequently observed in cancer cells has been an attractive target for pharmacological and molecular inhibition [18-21]. Normal tissue damage is a major cause of toxicity in cancer treatment, and the identification of targets that are cancer specific is critical to improve treatment outcome. In a recent study, Chk-α expression and the effect of its downregulation using small interfering RNA (siRNA) in human umbilical vein endothelial cells (HUVECs) relative to MDA-MB-231 human breast cancer cells were studied. The results indicated that transfection with Chk-siRNA in tumors reduced cancer cell proliferation, but did not affect endothelial cell proliferation during delivery, and further support the development of Chk-α downregulation as a cancer-specific treatment [22].

Targeting the tumor vasculature through antiangiogenic treatment is one of the attractive strategies to treat solid tumors. However, limited benefits utilizing VEGF targeting antibodies such as bevacizumab in breast cancer has been observed. It is now realized that subtype specific response to breast cancer treatment needs to be considered [23], and GPC was found to have a high potential as a biomarker for monitoring response to bevacizumab treatment, showing opposite response patterns in the basal and luminal like xenograft models. The phosphatidylinositol 3-kinase (PI3K) pathway is frequently activated in

cancer, and inhibitors targeting the PI3K pathway may represent a valuable treatment alternative. Predicting efficacy of these drugs is challenging, and methods for therapy monitoring are needed. Treatment with PI3K inhibitors resulted in significant growth inhibition in basal-like, but not luminal-like, xenografts. This indicates that PI3K inhibitors may have selective efficacy in basal-like breast cancer with increased PI3K signaling, and lactate, PCho and GPC were identified as potential metabolic biomarkers for early therapy monitoring [24].

Studies of the concerted actions between the gene expression and metabolic profile have led to mechanistically results suggesting that outcome in breast cancer can be determined by epigenetics and energy-metabolism[25], and that treatment of high risk cancer patients, identified by lactate/ketone gene signatures could be treated with targeted treatment towards the oxidative mitochondrial metabolism.

Functional consequences of mutations

Understanding the consequences of genetic alterations requires access to their functional effects at the phenotypic level, and MRS has emerged as a promising functional genomics probe. Mutations in the *isocitrate dehydrogenase* (*IDH*) genes are frequently found in gliomas, resulting in the production of the oncometabolite 2-hydroxyglutarate (2-HG) which can be observed with MRS both ex vivo [26,27] and in vivo [28,29]. IDH1 regulates several pathways towards lipid synthesis, and a distinct ^{31}P spectral pattern in IDH1 mutant glioma tumors have recently been identified [30]. Glioma patients harboring IDH mutations have a longer survival than their wild-type counterparts, and ^{31}P MRS may present an alternative prognostic biomarker for the identification of *IDH1*-mutated gliomas.

Disease-related mutations in the *MEN1* gene (encoding Menin protein) are responsible for the multiple endocrine neoplasia syndrome. Based on a cellular model allowing exogenous overexpression of either the wild type (WT) Menin protein or disease-related variant forms, several metabolites associated with the metabolic signature of pathogenic versus WT variants were observed [31]. Metabolic expressivity could possibly provide a simpler and more functional test for this cancer syndrome.

To conclude, metabogenomic approaches in cancer research can add useful information to the mechanistic understanding of metabolic profiles, stratify patients to treatment, identify new treatment targets and biomarkers for response to treatment and to understand functional consequences of mutations.

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