MOLECULAR ONCOLOGY 7 (2013) 130–142

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Subtype-specific response to bevacizumab is reflected in the metabolome and transcriptome of breast cancer xenografts

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ARTICLE INFO

Article history:
Received 15 August 2012
Accepted 15 October 2012
Available online 23 October 2012

Keywords:
Bevacizumab
Xenograft

ABSTRACT

Antiangiogenic therapy with bevacizumab has shown varying results in breast cancer clinical trials. Identifying robust biomarkers for selecting patients who may benefit from such treatment and for monitoring response is important for the future use of bevacizumab. Two established xenograft models representing basal-like and luminal-like breast cancer were used to study bevacizumab treatment response on the metabolic and gene expression levels. Tumor samples were obtained from mice treated with bevacizumab, doxorubicin or a combination of the two drugs, and high resolution magic angle spinning magnetic resonance spectroscopy and gene expression microarray analysis was performed.

Abbreviations: PFS, Progression Free Survival; OS, Overall Survival; FDA, Food and Drug Administration; tCHO, total Choline; MRS, Magnetic Resonance Spectroscopy; PCho, Phosphocholine; GPC, Glycerophosphocholine; HR MAS MRS, high resolution magic angle spinning magnetic resonance spectroscopy; SCID, Severe Combined Immuno Deficient; TSP, TrimethylSilyltetradeuteroPropionic acid; ERETIC, Electronic REference To access In vivo Concentrations; FE, Feature Extraction; IQR, Inter Quartile Range; GEO, Gene Expression Omnibus; ANOVA, Analysis Of Variance; FDR, False Discovery Rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

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1574-7891/$ – see front matter
http://dx.doi.org/10.1016/j.molonc.2012.10.005

Breast cancer
Transcriptomics
Metabolomics

Combination treatment with bevacizumab showed the strongest growth inhibiting effect in basal-like tumors, and this was reflected by a significant change in the metabolomic and transcriptomic profiles. In the luminal-like xenografts, addition of bevacizumab did not improve the effect of doxorubicin. On the global transcriptomic level, the largest gene expression changes were observed for the most efficient treatment in both models. Glycerophosphocholine showed opposite response in the treated xenografts compared with untreated controls; lower in basal-like and higher in luminal-like tumors. Comparing combination therapy with doxorubicin monotherapy in basal-like xenografts, 14 genes showed significant differential expression, including very low density lipoprotein receptor (*VLDLR*) and hemoglobin, theta 1 (*HBQ1*). Bevacizumab-treated tumors were associated with a more hypoxic phenotype, while no evidence was found for associations between bevacizumab treatment and vascular invasion or tumor grade.

This study underlines the importance of characterizing biological differences between subtypes of breast cancer to identify personalized biomarkers for improved patient stratification and evaluation of response to therapy.

1. Introduction

Targeting tumor vasculature has evolved as an attractive strategy to treat solid tumors. One implemented strategy for several cancers is antiangiogenic treatment utilizing a VEGF targeting antibody (bevacizumab) (Braghiroli et al., 2012). Limited benefit in progression free survival (PFS) and lack of benefit in overall survival (OS) reported from clinical trials have raised questions about the relevance of bevacizumab for advanced breast cancer (Burstein, 2011). In 2010, FDA has revoked the breast cancer indication for bevacizumab. However, recent results have indicated that prolonged bevacizumab administration in metastatic breast cancer patients may give a benefit in OS (Bear et al., 2012; Smith et al., 2011; Von Minckwitz et al., 2012). The clinical utility of bevacizumab in breast cancer will depend on the identification of subgroups of patients who are likely to benefit from antiangiogenic therapy (Schneider and Sledge, Jr. 2011). Several potential biomarkers have been proposed, including VEGFR polymorphisms, and VEGF, PDGFR-β and VCAM1 expression (Schneider et al., 2008; Yang et al., 2008; Jubb et al., 2011; Baar et al., 2009), but none have been established as reproducible. Hence, the aim of this study was to investigate the biology of treatment response and possible resistance effects by combining metabolomics and transcriptomics of breast cancer xenograft models in order to identify response biomarker candidates for later verification in a clinical setting.

The tumor metabolome is known to be highly affected by extracellular factors such as the microenvironment, pH, oxygen, nutrients and drugs. Thus, metabolomic techniques have the potential to be more sensitive in monitoring treatment than other approaches such as measuring levels of RNA or proteins. Several metabolomic markers of treatment response have been suggested, including total choline (tCho), measured using *in vivo* MRS (Jagannathan et al., 2001; Meisamy et al., 2004), and phosphocholine (PCho), glycerophosphocholine (GPC) and lactate, measured using higher resolution MRS (Beloueche-Babari et al., 2010; Podo et al., 2011). High PCho levels or high PCho/GPC ratio with corresponding expression levels of genes involved in choline metabolism have been associated with malignancy and aggressiveness in both triple negative and ER positive breast cancer cell lines (Eliyahu et al., 2007; Glunde et al., 2004; Katz-Brull et al., 2002). On the other hand, high levels of GPC have been associated with ER negative tumors in studies of human breast carcinomas (Barzilai et al., 1991; Giskeodegard et al., 2010), suggesting that *in vitro* studies do not capture the complexity of tumor metabolism.

In vivo models are valuable tools for studying treatment response mechanisms since human carcinomas can be studied surrounded by a relevant microenvironment (Vargo-Gogola and Rosen, 2007). Two directly grafted orthotopic xenograft models representing basal-like and luminal-like breast cancer have previously been established and characterized at the transcriptomic and metabolomic levels (Bergamaschi et al., 2009; Lindholm et al., 2012; Moestue et al., 2010). The luminal-like model had a high PCho/GPC ratio while the basal-like model showed the opposite. The same differences were also found in clinical tumor samples, suggesting that these two models are relevant for studies of metabolism and treatment response in these two types of breast cancer (Moestue et al., 2010).

Recently, treatment studies in these models demonstrated that the basal-like model showed significantly improved response to bevacizumab and doxorubicin in combination compared with doxorubicin alone, while the luminal-like model responded equally well to doxorubicin with or without antiangiogenic therapy (Lindholm et al., 2012). Metabolomic and transcriptomic analysis of tumor tissue from these experiments was performed using high resolution magic angle spinning magnetic resonance spectroscopy (HR MAS MRS) and gene expression microarrays.

We demonstrate that GPC is a promising biomarker on the metabolomic level and that several gene transcripts are associated with bevacizumab responses in the responding basal-like tumors.