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Article

A Gene Signature Predictive for Outcome in Advanced Ovarian Cancer Identifies a Survival Factor: Microfibril-Associated Glycoprotein 2

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SUMMARY

Advanced stage papillary serous tumors of the ovary are responsible for the majority of ovarian cancer deaths, yet the molecular determinants modulating patient survival are poorly characterized. Here, we identify and validate a prognostic gene expression signature correlating with survival in a series of microdissected serous ovarian tumors. Independent evaluation confirmed the association of a prognostic gene microfibril-associated glycoprotein 2 (*MAGP2*) with poor prognosis, whereas in vitro mechanistic analyses demonstrated its ability to prolong tumor cell survival and stimulate endothelial cell motility and survival via the $\alpha_V\beta_3$ integrin receptor. Increased MAGP2 expression correlated with microvessel density suggesting a proangiogenic role in vivo. Thus, MAGP2 may serve as a survival-associated target.

INTRODUCTION

Ovarian cancer is the fifth most common form of cancer in women in the United States, accounting for 3% of the total number of cancer cases and for 26% of those cases occurring in the female genital tract. Epithelial ovarian cancer is highly lethal. In 2009, there will be an estimated 21,550 new cases and 14,660 deaths from ovarian caner in the United States (Jemal et al., 2009). Of these deaths, the vast majority will be attributed to papillary serous tumors, which account for 60% of all cases (Boring et al., 1994). Most of these tumors are detected at an advanced stage with metastases present beyond the ovaries precluding curative treatment (Boente et al., 1996). Clinical management of this disease involves tumor debulking followed by administration of carboplatin chemotherapy. Despite the fact that 80% of advanced ovarian cancers (stages

SIGNIFICANCE

Ovarian cancer is the most lethal gynecologic cancer in the United States, yet little is known about the molecular events impacting survival. Thus, efforts to stratify patients for therapy and identify therapeutic targets have been severely hampered. We have now identified and confirmed a gene expression signature correlating with poor survival in microdissected advanced serous ovarian tumors. In addition, we have completed an initial characterization of a proangiogenic factor associated with prognosis, MAGP2, which can promote tumor epithelial cell survival and stimulate endothelial cell motility and survival. Correlation of MAGP2 with microvessel density suggests a role in neovascularization in vivo. This work has developed a prognostic gene signature of biological significance and identified a putative ovarian cancer target.

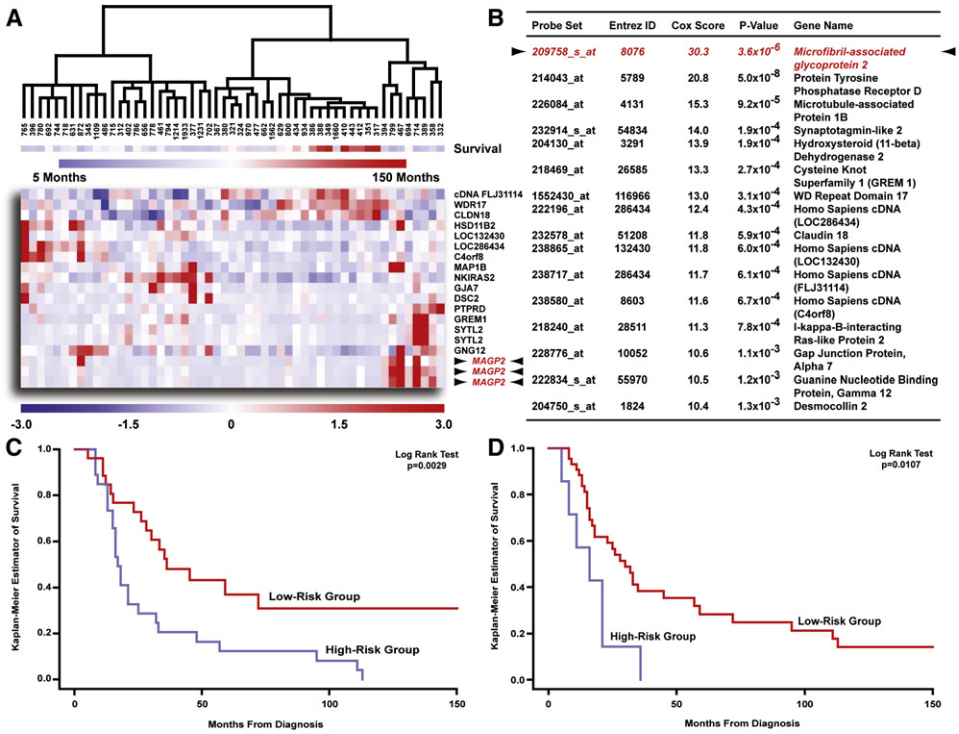

Figure 1. Identification and Validation of a Prognostic Gene Expression Signature Correlating with Survival in 53 Microdissected Late-Stage High-Grade Papillary Serous Ovarian Tumors

(A) Hierarchical clustering of 53 advanced stage, high-grade serous adenocarcinomas using expression values for genes possessing a Cox score >10 (gene expression: red, upregulated; blue, downregulated; survival: blue, short survival; red, long survival).

(B) Genes presented in this table possessed a large Cox score (>10). Only the probe set with the highest Cox score is presented for *MAGP2*.

(C) Kaplan Meier analysis of the predictor demonstrated a significant difference in survival time (p = 0.0029).

(D) Kaplan Meier survival analysis of 49 patients using qRT-PCR validation data obtained for the top 11 survival-signature genes confirmed the two groups retained significantly different survival endpoints (p = 0.0107).

III and IV) respond to primary treatment with surgery and chemotherapy, the disease usually recurs and is ultimately fatal. A subset of these patients will develop a more chronic form of ovarian cancer and may survive 5 years or more with treatment. Consequently, there is a pressing need for diagnostic classifiers that can reliably stratify patients for therapy, as well as targets for therapeutic intervention.

Previous studies have shown that large-scale transcription profiling can identify differentially expressed genes and molecular signatures in numerous biological systems including ovarian cancer (Alizadeh et al., 2000; Bonome et al., 2005; DeRisi et al., 1997; Golub et al., 1999). More recent efforts to derive clinical predictors for survival in ovarian cancer from gene expression data have focused on discrete patient groups clustered at either end of the survival spectrum (Berchuck et al., 2005; Lancaster et al., 2004). Yet, expression patterns identified in this manner may not adequately differentiate the majority of patients who will succumb at an intermediate endpoint. In addition, the evaluation of undissected tumor isolates may introduce erroneous data attributable to varying amounts of intervening stroma and lymphocytic infiltrate. In this study, we describe the use of a whole-genome oligonucleotide array to perform expression profiling on a series of microdissected late-stage, high-grade papillary serous ovarian adenocarcinomas to identify a prognostic gene signature correlating with survival as a continuous variable.

RESULTS

Derivation and Validation of a Gene Signature Predictive for Survival in Patients with Advanced Papillary Serous Ovarian Cancer

We identified 53 advanced stage, high-grade primary tumor specimens from patients with papillary serous adenocarcinomas of the ovary whose survival spanned a spectrum of 145 months (see Table S1, available online). The average age for the cohort was 61.9 years (SD = 12.7), with an average survival time of 40.5 months following surgery (SD = 41.3 months). Among these patients, 12 were still alive when we analyzed the data and 11 patients were suboptimally debulked. All specimens were subjected to laser-based microdissection and analyzed as pure, microdissected epithelial cell populations on whole-genome Affymetrix U133 Plus 2.0 GeneChip microarrays.

To derive the predictor, we used a two-step ''semi-supervised'' approach using Cox regression analysis and leave-one-out cross-validation to identify and validate the survival signature. The performance of the prediction analysis was visualized by hierarchical clustering, which demonstrated the ability of the top scoring genes (Cox hazard ratio >10) to cluster the 53 specimens according to survival (Figure 1A). As detailed in Figure 1B, the gene possessing the highest hazard ratio was *MAGP2*. Finally, the validity of the entire 200 probe set classifier