

CHARACTERIZATION OF GENE EXPRESSION SIGNATURES OF TUMOR IMMUNOGENICITY AND CELLULAR PROLIFERATION FROM MURINE CANCER MODELS GROWN *IN VITRO* AND *IN VIVO*

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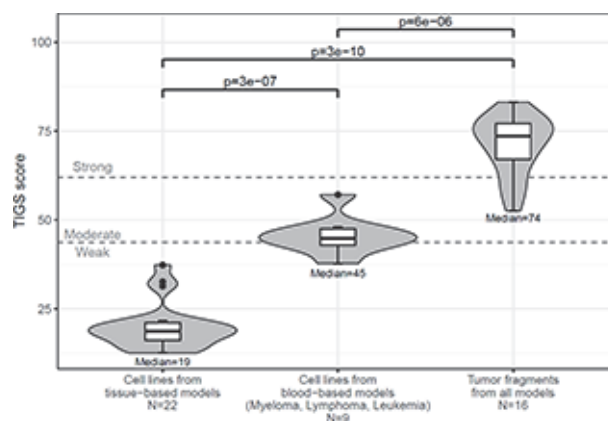
Background Tumor immunogenicity and cellular proliferation are critical factors influencing cancer progression and response to therapy. The precise way these gene expression signatures change across cancer types and model systems remains incompletely understood, and, in particular, the absence of immune infiltration and vascularization *in vitro* may result in differential gene expression. Our study aimed to characterize gene expression profiles from different murine tumor cell lines *in vitro* and *in vivo*, comparing cell lines grown in culture from tissue-based malignancies (CT), cell lines grown in culture from blood-based malignancies (CB), and tumor fragments collected from syngeneic models (TF).

Methods Our analysis included a total of 37 cell lines of various cancer types from 13 different organ sites. Cell lines were expanded in cell culture, while tumor fragments were harvested from mouse tissues. RNA sequencing (RNA-seq, 474 genes) was performed to capture gene expression changes, and we developed signatures related to tumor immunogenicity (TIGS, 146 genes) and cellular proliferation (CP, 9 genes including Ki-67), based on the gene expression ranks relative to values abstracted from the literature in pan-tumor assessments. Samples were categorized into three groups: CT (n=22), CB (n=9), and TF (n=16). There were 10 samples from the TF cohort that were derived from cell lines included in the CT cohort. Hierarchical clustering was employed using immune gene expression ranks. Statistical differences were evaluated using Wilcoxon rank-sum test with Bonferroni multiple testing correction.

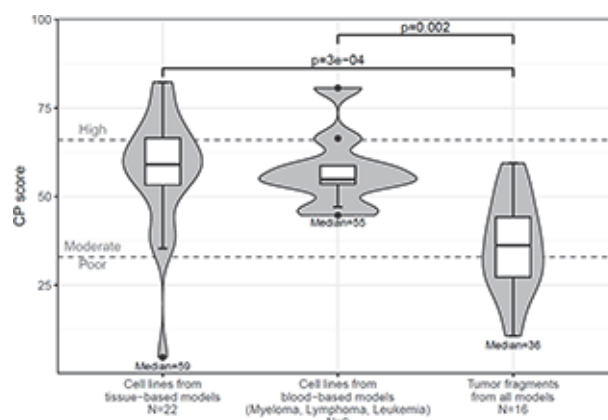
Results CT samples exhibited weak TIGS (median 19; figure 1) and high CP (median 59; figure 2). CB samples displayed moderate TIGS (median 45) and high CP (median 55). In contrast, TF samples demonstrated high TIGS (median 74) and low CP (median 36). Statistical comparisons demonstrated that TF samples were significantly more immunogenic than both CT ($p < 0.0001$) and CB ($p < 0.0001$) samples. Furthermore, TF samples exhibited significantly reduced CP signature, as compared to both CT ($p < 0.0001$) and CB ($p < 0.0001$) groups.

Conclusions Our findings demonstrate that tumor tissues collected *in vivo* exhibit a higher TIGS signature (correlating with the absence of immune cells in cell culture models) and lower CP signature than cell lines grown in culture. These results support the notion that an active immune system and a vascularized environment contribute to decreased proliferation of cancer cells through immune activation. Additionally, the marked differences in gene expression profiles between *in vivo* tumor fragments and *in vitro* cell lines highlight the importance of utilizing syngeneic models for studying cancer therapeutics, particularly immune-based therapeutic agents.

Ethics Approval Ethics approval for this study was obtained from WCG IRB (Study #1340120), an independent institutional review board, including waiver of informed consent



Abstract 1455 Figure 1 Comparison of TIGS gene signature between different cohorts



Abstract 1455 Figure 2 Comparison of CP gene signature between different cohorts

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