SKIN

BRIEF ARTICLE

Cellular Deconvolution Reveals Unique Findings in Several Cell Type Fractions Within the Basal Cell Carcinoma Tumor Microenvironment

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ABSTRACT

Introduction: Despite therapeutic advancements, locally advanced and metastatic basal cell carcinomas continue to carry poor prognoses and high recurrence rates. Current treatment options remain suboptimal due to limited efficacy and associated adverse events. The objectives of this study are to 1) characterize the basal cell carcinoma immune cell microenvironment and 2) identify novel therapeutic targets.
Methods: Bulk expression data for 25 basal cell carcinoma and 25 control tissue samples was obtained from the Gene Expression Omnibus. Cell type fraction estimates were derived by least-squares deconvolution. Population differences were determined by Mann-Whitney U test.
Results: Most significantly, two deconvolution algorithms similarly observed greater B cell infiltration in tumor samples compared to normal tissue (P<0.0001).
Conclusion: Importantly, the results of this study provide new insight into the basal cell carcinoma tumor microenvironment and nominate testable immune cell populations for future therapeutic discovery. Study limitations include sample size and applicable background prediction levels of bulk deconvolution tools.

INTRODUCTION

While most cutaneous basal cell carcinomas (BCC) are amenable to surgery and other local therapies, inoperable and aggressive BCC require more specialized care. Novel molecular therapies, such as vismodegib and sonidegib, have been developed to target the aberrant hedgehog (HH) signaling found in BCCs; however, these agents are associated with side effects that significantly hinder medication adherence, emphasizing the need for additional targeted therapies and enhanced understanding of the tumor microenvironment.[1] Undoubtedly, in silico analysis of the BCC tumor microenvironment can provide critical insight into tumor behavior, help identify additional therapeutic targets, and facilitate the development of superior immunotherapies.

Transcriptome deconvolution is a computational technique that utilizes expression data from an RNA sample to estimate its cellular composition. Although cell flow cytometry can similarly quantify cell types, deconvolution is time-efficient, cost-effective, and can be performed on existing data.

METHODS

Bulk RNA-seq data for 25 BCC and 25 adjacent normal tissue samples was retrieved from the Gene Expression Omnibus (accession ID GSE125285).[2] Processed log2(TPM+1) counts were fed into the quanTIseq and EPIC deconvolution pipelines via R package 'immunedeconv' v2.2.1.[3] As a validation measure, these tools were used because they both rely on constrained least squares regression to estimate cell type fractions relative to the total cell population. Cell fraction differences between tumor and nontumor tissue samples were determined by Mann-Whitney U test. Statistical significance was set at $P<0.05$.

RESULTS

Higher levels of monocytes, non-regulatory CD4+ T cells and Tregs, in addition to lower levels of myeloid dendritic cells and neutrophils were resolved by quanTIseq. A lower endothelial cell fraction in the tumor set was resolved by EPIC ($P<0.01$). Additionally, EPIC expectedly indicated significantly higher rates of cancer-associated fibroblasts in the tumor population, thereby validating the procedure. Consensus between pipelines was achieved in the assessment of B cells, wherefor higher levels were reported in the tumor samples ($P<0.0001$). **Table 1** summarizes these findings.

DISCUSSION

Compared to healthy tissue, EPIC revealed significantly increased B cells and cancer-associated fibroblasts and significantly reduced endothelial and NK cells in the BCC tumor microenvironment, whereas quanTIseq revealed significantly increased B cells, non-regulatory CD4+ T cells, CD8+ T cells, monocytes, Tregs, uncharacterized cells with significantly reduced myeloid dendritic cells and neutrophils. Consensus was achieved for B cells, as both deconvolution tools revealed increased B cells in the tumor microenvironment.

An investigation of 22 BCCs from 18 patients using monoclonal antibodies observed a predominantly T-cell mediated response in the peritumoral infiltrate with minor B cell participation.[4] However, a recent CIBERSORTx deconvolution analysis reported greater B lymphocyte infiltration in non-advanced BCCs compared to advanced BCCs.[5] Lefrançois and colleagues state that while lymphocytic infiltration in non-advanced carcinomas may depict an adaptive anti-tumor response, IL-10-producing B cells have also been implicated in tumor-associated macrophage proliferation, suggesting potential anti and pro-tumorigenic properties of B cells in the tumor microenvironment.[5] Moreover, their results indicated greater B cell infiltration among vismodegib-sensitive basal cell carcinoma samples ($P<0.00001$),[5] supporting a role for tumor microenvironment analysis in predicting chemotherapy efficacy.

Our study is limited by sample size. In addition, transcriptomic deconvolution tools are limited by their respective true background prediction levels, as described by Sturm and colleagues.[6] Yet, using three criteria (score interpretability, overall

Table 1. Mean fraction of distinct cell types, relative to total cell population, in basal cell carcinoma tumor and adjacent normal tissue samples.

Cell type	EPIC			quanTIseq		
	Normal (n=25)	Tumor (n=25)	*P*-value	Normal (n=25)	Tumor (n=25)	*P*-value
B cell	0.87%	1.74%	**1.33E-05**	2.73%	4.24%	**9.82e-05**
Cancer-associated fibroblasts	1.48%	1.69%	**5.53E-04**	-	-	-
CD4+ T cell	19.13%	17.83%	7.09E-02	-	-	-
CD4+ T cell (Non-regulatory)	-	-	-	0.00%	3.23%	**7.61e-06**
CD8+ T cell	7.85%	7.92%	7.88E-01	0.04%	0.34%	**1.60e-05**
Endothelial cell	12.61%	11.04%	**1.03E-03**	-	-	-
Macrophage (Total)	0.61%	0.56%	1.41E-01	-	-	-
Macrophage M1	-	-	-	0.30%	0.60%	1.31e-01
Macrophage M2	-	-	-	2.14%	2.71%	2.44e-01
Monocyte	-	-	-	0.29%	4.45%	**3.50e-06**
Myeloid dendritic cell	-	-	-	2.65%	1.73%	**3.78e-02**
Neutrophil	-	-	-	31.97%	15.98%	**2.39e-06**
NK Cell	2.8E-08%	1.3E-07%	**2.61E-02**	1.05%	1.14%	4.88e-01
Regulatory T cell (Tregs)	-	-	-	1.04%	1.55%	**9.86e-03**
Uncharacterized cell*	57.43%	59.21%	2.31E-01	57.78%	64.02%	**2.89e-02**

*Proportion of cells not accounted for by the signature matrix. In RNA-seq data from bulk tumor, the 'uncharacterized' fraction represents the tumor content.

performance, and possible limitations), the authors recommended EPIC for analysis of B cells, CD4+ T cells, CD8+ T cells, NK cells, macrophages and monocytes, fibroblasts, and endothelial cells, and quanTIseq for analysis of non-regulatory and regulatory CD4+ T cells and CD8+ T cells.[6]

Overall, this analysis provides new insights into the basal cell carcinoma tumor microenvironment, introducing the potential significance of B cell infiltration, among other important findings.

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