Research Article

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Systematically exploring the effect of tumor mutation and cytokines/chemokines on colon adenocarcinoma (COAD)

We first estimated immune cell composition in 458 COAD tumors from TCGA, and then ev mut M significantly higher immune cell infiltrates. Gene expression of IFNG, TGFB1, TNF, IL6, IL10, CX3CL1, CXCL9, CXCL10 were all positively correlated with immune cell infiltrates, and inversely correlated with purity (P<0.05 after Bonferroni correction) in tumor specimens. In survival analysis, none of these chemokin e chemokin e chemokin P=0.023; B cell: HR=135.38, 95% CI=5.27-3480.28, P=0.003).

Our results suggest that genetic mutation and chemokines/cytokines were correlated with infiltration of immune killer cells and that the mutation status and inflammation biomarker expression levels could be used to select patients for

Proportions of immune cell subsets were estimated in 458 COAD tumors from TCGA and the relationship between immune cell subsets, chemokines, and cytokines and patient survival was systematically assessed. Our study revealed significant biomarkers for tumor immune response and CRC progression.

COAD; Genetic mutations; Cytokines/chemokines; Immune response; Outcomes

Introduction

Colon cancer is the third most common cancer with a high mortality worldwide. The prognosis of advanced patients is still very poor. The process of tumor development and progression is determined by two factors, genetic/epigenetic changes in the tumor cells and the interactions between the cell elements in the tumor microenvironment (TME). TME consists of different types of cells, including tumor, stromal, immune, and endothelial cells. Tumor-infiltrating lymphocytes (TILs) play a key role in anti-tumor immunity and therapy elicited response in patients with solid tumors including colon cancer and other cancers [1-4]. RNA sequencing (RNA-seq) deconvolution procedures can estimate cellular fractions and functions of infiltrating immune cells in TME and can help to evaluate their roles in patient progression [5-8].

Genetics has a key role in predisposition to colon adenocarcinoma (COAD) and in its initiation and progression. Neoantigens generated by somatic mutations in tumor cells can be recognized by host CD8+ and CD4+ T cells. Previous experimental studies used identified antigens in COAD cell lines to successfully induce downstream immune reactions [9,10]. High tumor mutation burden is an emerging biomarker for response to immunotherapy in several types of cancer [11,12]. In 2017, FDA approved pembrolizumab as the immune checkpoint therapy for a high mutation load COAD with DNA mismatch repair deficient or with elevated microsatellite instability. However, for those COAD tumors with low mutation load that had low response to immune checkpoint therapy, further eva

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roles of germline and somatic mutation as potential determinants of immunogenicity in these subsets is essential.

Cytokines and chemokines play critical roles in regulating innate and adaptive immune responses and cell-cell interactions. Tumor neoantigens are recognized as foreigners to induce anti-tumor responses such as higher TIL density and increased expression of type II interferon (IFN-γ)(IFNG) related genes, for example PD-L1 and CTLA-4. A clinical study show that increased tumor IFNG gene expression predicts a better clinical outcome among multiple tumor types [13]. Furthermore, TGF-β led to enhanced activin secretion and a higher combined activin/TGF-β ligand expression score was associated with a shorter disease free survival in patients with

oncogenes contribute to antitumor immune response. These mutated genes may be important biomarkers for tumor progression.

Chemokines/cytokines related to immune cell infiltrates in COAD tumors

We applied TIMER 2.0, EPIC and CIBERSORT-ABS deconvolution tools to estimate immune cell proportions and tumor purity based on RNA-seq

infiltrates	APC(mutated n=286)			TP53(mutated n=216)			TTN(mutated n=207)			KRAS(mutated n=174)		
	log2FC(Mutated/Wild)	p	adj.p	log2FC(Mutated/Wild)	p	adj.p	log2FC(Mutated/Wild)	p	adj.p	log2FC(Mutated/Wild)	p	adj.p
T cell CD8+_TIMER	-0.362034196	0.000732964	0.036543499	-0.133364512	0.075708612	0.289833979	0.255684781	0.001348312	0.043707779	-0.060916297	0.656740297	0.865703119
T cell CD4+_TIMER	-0.188952851	0.53517918	0.930530153	0.061777697	0.57129971	0.825363284	0.099183781	0.093355297	0.453545023	-0.087931747	0.82601536	0.963742483
B cell_TIMER	-0.498784644	0.001508677	0.263515627	-0.494472435	1.94E-05	0.000524711	0.089923945	0.310041945	0.788734871	-0.119652929	0.70069594	0.863152216
Macrophage_TIMER	-0.435130972	0.196181902	0.727681808	0.289000827	0.20835934	0.459777294	0.218942449	0.344572086	0.675886646	-0.410582862	0.06792074	0.374305793
Neutrophil_TIMER	-0.351072434	1.57E-05	0.003271979	-0.081931391	0.0937461	0.347321617	0.370302473	6.28E-06	0.001457548	-0.13832713	0.042298957	0.324081009
Myeloid dendritic cell_TIMER	-0.309708852	9.65E-06	0.002416206	-0.128536755	0.003945176	0.043977113	0.263905725	2.27E-06	0.000542758	-0.097088014	0.062894261	0.501581731
NK cell_EPIC	-1.897606437	0.000649749	0.045482462	-0.812336562	0.79122816	0.972448927	2.917046669	1.07E-05	0.001666583	-0.873366702	0.050809225	0.591234619

Myeloid dendritic cell_TIMER	0.180946181	0.008527906	0.215523441	0.202528578	0.01046795	0.289076472	0.488521471	1.08E-08	4.92E-07	0.119136185	0.066703412	0.785281074
NK cell_EPIC	1.621902093	0.020230395	0.469425978	2.004444759	0.001105217	0.277039587	3.03449155	2.01E-07	1.71E-05	1.109534942	0.309605867	0.904572894
fibroblast_EPIC												

infiltrates	**MUC4(mutated n=32)**			**PTEN(mutated n=24)**			**KIT(mutated n=21)**			**TGFBR2(mutated n=18)**		
	log2FC(Mutated/Wild)	**p**	**adj.p**	**log2FC(Mutated/Wild)**	**p**	**adj.p**	**log2FC(Mutated/Wild)**	**p**	**adj.p**	**log2FC(Mutated/Wild)**	**p**	**adj.p**
T cell CD8+_TIMER	0.355914318	0.016535124	0.373696527	0.059673921	0.387764399	0.914691574	0.229819551	0.085716668	0.466028279	-0.054053027	0.967633341	0.994289133
T cell CD4+_TIMER	0.084902884	0.668289024	0.958927201	-0.048628625	0.774819694	0.976425943	-0.195492049	0.484444119	0.864398632	0.04380454	0.344276675	0.858966887
B cell_TIMER	0.423691904	0.030211212	0.824603704	-0.056558203	0.993477939	1	-0.105958241	0.468241856	0.8947244	0.210082434	0.06506219	0.797021093
Macrophage_TIMER	0.198578541	0.276483446	0.745014245	-0.162106287	0.551003023	0.884553347	-0.235325637	0.501459631	0.811024963	0.297426054	0.940246646	0.99598644
Neutrophil_TIMER	0.511530945	0.000181411	0.021761463	0.224296257	0.099655109	0.514435834	0.348688419	0.008553766	0.338382301	0.12376846	0.142658417	0.903929021
Myeloid dendritic cell_TIMER	0.368077546	0.00182615	0.19984709	0.17092632	0.189412794	0.645287339	0.285201735	0.008700848	0.153032565	0.16844723	0.179884262	0.701708984
NK cell_EPIC	2.807536823	0.000102961	0.009609687	1.372131079	0.019358211	0.309731377	1.941173767	0.002780049	0.19569358	0.776073267	0.035857211	0.343607109
fibroblast_EPIC												

Association between tumor mutation and immune cells among 404 COAD samples.

data for the COAD patients in TCGA[6-8]. We then evaluated correlations of chemokine and cytokine gene expression with T cell abundance and tumor purit16-6 (i)1 92 (-AB (b)11 k)-1.[illegible]79 tn(l)12 7in he Cyto1t16-65.oo( )]TJ0.106002 0 -1.2 Td[(a)92 7iithioytod cc (a)19 (t

CTLA4

Tumor purity

Pr

CX3CL1	0.49	1.58E-28	0.42	4.01E-21	-0.28	7.44E-09	0.35	3.64E-09	1.22	1.03-1.45	2.00E-02
	0.71		0.7		0						

Spearman correlation test.

Purity-corrected partial Spearman correlation.

HR: hazards ratio; CI: confidence interval

Relationship between cytokine or chemokine gene expression levels and tumor immune response or overall survival in 458 patients with CRC whose sequencing data were available in The Cancer Genome Atlas.

Relationship between cytokine or chemokine gene expression levels and other immune cells estimated with TIMER in 458 patients with CRC whose sequencing data were available in The Cancer Genome Atlas.

the fraction of immune cell subsets, Li et al discovered chemokine–receptor networks for lymphocyte infiltration in several tumors. In that research, CD8+ T cell levels were found to be correlated with abundance of chemokine–receptor pairs, including CCL3,4,5–CCR1,5 and XCL1,2–XCR1, and macrophage subset was associated with the CXCL12–CXCR4 pair in head and neck, thyroid, stomach, and colon cancers [4]. Among selected chemokines and cytokines related to tumor inflammation and immune response, we discovered that several cytokines were associated with CD8+ T cell enrichment, indicating that these biomarkers could be potentially targeted to boost CD8+ T cell responses, or to select patients more likely to respond to immunotherapy.

The current study has some limitations. TCGA database has limited information on clinical annotation, detailed pathology information, prior treatment data and sufficient survival outcome data in the patient cohort, which had prevented us from exploring potential roles of systemic therapy, including immunotherapy, in the patients used in the current study. In the current study, the association between tumor T-cell subsets and survival outcome was not significant in the COAD, and the association between CD4+ and B cell and COAD outcome was significant but had a wide confidence interval, which could be due to smaller sample size in the tumor cohort, or due to the smaller number of events among patients who provided tumors (277 samples with only 67 dying). Previouiene co8+ T celpes showed that elevated CD8+ T cell subsets predicted reduced recurrence in colorectal cancer [20,38]. Another study using TCGA data showed that CD4+ T cell related genes were correlated with OS, but no CD8+ T cell related genes were found to be associated with OS in COAD [39]. These findings in TCGA were consistent with our results. Another potential limitation of this study was the curse of data dimensionality problem the small number of samples with respect to the large features of gene expression data. Finally, we should recognize that the CD8+ T-cells population itself evolves over time and contains heterogeneous components; evaluating the relative roles of CD8+ T-cell subsets is essential but beyond the scope of the current study.

We assessed immune-cell populations based on RNA-seq from single time point tumor tissues using TIMER platform; the approach can't discern stromal or intra-tumor immune-cell localization or consider tumor heterogeneity or different metastatic tumor sites (for example, solid organ vs. lymph node). Further investigations that covered data with accurate spatial and temporal data could help resolve these problems. We recogacA2 (ra)-5ol 1036 Tw 0 -1.2 Tde that thr resui(r r)13 (p)-9 (o)12 urte (h)4 (r)13 in,er i4-9

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