Chemokines, Chemokine Receptors, and Tumor-infiltrating Lymphocytes in Melanoma

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BACKGROUND

Melanoma, the most aggressive form of skin cancer, serves as the prototypical *immunogenic malignancy*.

Presence of tumor-infiltrating lymphocytes (TILs) is known to be a favorable prognostic factor, through recognition and disruption of cancer cells.

Chemokines (**CK**) are a family of cytokines that interact with G-protein coupled receptors (**CKR**) to regulate the movement of white blood cells, specifically T and B cells. Chemokine axes such as *CCL2-CCR2*, *CCL5-CCR5*, and *CXCL12-CXCR4* normally recruit immune cells to tissues, but these axes can also be manipulated by melanoma cells to promote immune evasion and tumor progression.

We hypothesize that inherited gene variants in CK/CKR genes may modulate these interactions and contribute to different degrees of lymphocytic infiltration in melanomas.

This study **aims to determine associations between inherited gene variants in chemokine genes and presence of TILs** in primary melanoma.

METHODS

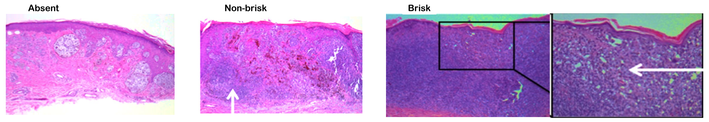
Cohort. Patients with incident primary skin melanoma were recruited through the international multi-center population-based *Genes, Environment, and Melanoma (GEM)* study, and provided demographic, clinicopathologic, and detailed sun-exposure data plus buccal samples for genetic studies. Individuals of Caucasian/European descent and contributed biospecimens to 9 study-centers internationally.

Centralized pathology review. TILs data were available for 2,610 melanomas. TILs were categorized as absent / non-brisk (scattered infiltration) / brisk (widespread infiltration) (see figure).

Genetics data were available for 221 candidate CK and CKR gene variants, mainly selected based on previously published associations with melanoma, other cancers, chronic inflammation, and known or predicted functional relevance

Statistical Analysis. Associations between genotypes and TILs were assessed using multinomial logistic regression, including age at diagnosis, sex, age-by-sex interaction, and study center in the model. Additive, dominant, and recessive inheritance models were considered.

Other. For nominally significant associations ($p \leq 0.05$) SNPs were further evaluated for their functional implications using public databases: Genotype-Tissue Expression (GTEx, effect of tested SNPs on downstream gene expression). DICE and Atlas were mined to evaluate gene expression at the tissue and single cell level.



Melanoma sections stained with hematoxylin & eosin showing TIL categories.

RESULTS

Nominally significant associations

- 19 SNPs across 11 CK/CKR genes (*DARC, CXCR1, CXCR2, CXCL12, CXCL16, CCL2, CCL4;CCL3, CCR5, CCL7, CCL8, CCL11*) were nominally significantly ($p < 0.05$) associated with brisk- and non-brisk TILs.
- Effect sizes were calculated using patients within the GEM cohort who lacked TILs as the reference group.
- The per allele Effect Size for non-brisk TILs ranged from 26 to 30%, whereas for brisk TILs, it ranged from 33 to 56%.
- The effect sizes ranged from 46 to 72% (recessive) and 51 to 56% (dominant) models for Brisk TILs.
- The effect sizes ranged from 41 to 50% (recessive) and 29 to 37% (dominant) models for Brisk TILs.

Summary of Effect of CK/CKR Alleles on Gene Expression

- 3 variants + 53 proxies affect gene expression of *CCL4*, *CXCL6*, *CCR1*, *CCR2*
- Target gene: ***CCL4***
  - rs1719153*T (and proxies) is associated with brisk TILs & increased *CCL4* in whole blood and skin (sun exposed or unexposed)
  - *CCL4* is a favorable prognostic factor in melanoma TCGA
- Target gene: ***CCR1***
  - *CCR5* rs2734648*T is associated with non-brisk TILs & decreased *CCR1* in sun exposed skin – only
  - *CCR5* is associated with recruitment of immunosuppressive cells and is associated with melanoma progression and metastasis.
  - When/if CCR1 is activated (*CCL3*, *CCL5-9*, *CCL13-16*, *CCL23*), signaling leads to migration of immune cells towards the source of the chemokine
- Target gene: ***CCR2***
  - *CCR5* rs2734648*T (and proxies) is associated with non-brisk TILs & increased *CCR2*
  - *CCR2* is key for *CCL2*; mediates recruitment of immune cells to the tumor, promoting or suppressing tumor growth; expression on melanoma cells can enhance their migration, invasion, and metastasis.
- Target gene: ***CXCL16***
  - *CCXL16 gene expression is downregulated by rs1050998*G and its proxies in whole blood and skin; this SNP is significantly associated with the Brisk Tm. Infiltrating lymphocytes.*
  - *CXCL16*, the ligand of *CXCR6*, is part of the pathway associated with migration of tumor cells and more aggressive cancer stem-cells (CSCs) in melanoma; it is also highly expressed in the unique metastatic tumor microenvironment (TME).

Effect of significant CK/CKR variants on gene expression in skin and whole blood

Affected Gene	Type of effect induced by alleles and correlated proxy CK/CKR alleles	Reported expression in epidermis, melanocytes, melanoma, & immune cells	Reported association or function
CCL4	Upregulation	T cells only	The protein encoded by this gene is enriched in melanomas tissues and recruits myeloid-derived suppressor cells (MDSCs) which produce an immunosuppressive effect.
CCR1	Downregulation	T cells only	*CCR1* signaling, driven by chemokines *CCL8* and *CCL15*, promotes melanoma survival and proliferation.
CCR2	Upregulation	monocytes, peripheral blood mononuclear cells (PBMCs)	When engineered in T cells via TCR, the protein encoded by this gene displays anti-tumor effects in melanoma models by enhancing migration of TILs to tumor sites. Additionally, this protein has been identified as a potential therapeutic target, as classical monocytes have been shown to lose *CCR2* and differentiate, displaying anticancer properties; an increase in expression during inflammatory states was also observed by *CCR2*-expressing nonclassical monocytes.

Effect of CK/CKR alleles & their proxies on target genes expression in skin and whole blood

Variants in GEM with effect on GEx		Effect of variants (n) Proxies	Affected Gene	Direction of Effect on Gene Expression and Association p-value (range) Skin - Sun Exposed		Skin - Not Sun Exposed		Whole Blood	
CCL4;CCL3	rs1719153	32	CCL4	Upregulation	1.62E-09–2.76E-14	Upregulation	1.29E-06–2.54E-10	Upregulation	9.67E-05–1.07E-05
CCR5	rs2734648	14	CCR1	Downregulation	2.28E-05–6.12E-07	–	–	–	–
			CCR2	Upregulation	1.32E-07–2.76E-08	Upregulation	5.29E-05–3.54E-07	Upregulation	6.00E-05–1.64E-05
CXCL16	rs1050998	7	CXCL16	Downregulation	1.48E-34–8.02E-35	Downregulation	4.79E-08–8.55E-09	Downregulation	3.15E-12–8.44E-13
			ZMYND15	Downregulation	3.82E-29–3.24E-30	Downregulation	9.44E-10–3.33E-10	Downregulation	3.21E-05–1.37E-05
			MED11	Downregulation	6.19E-15–3.16E-15	–	–	–	–

DISCUSSION

Our findings suggest that the complex and dynamic relationship between CKs, CKRs, and TILs is modulated by inherited polymorphisms, which both directly and indirectly affect the migration of immune cells via several chemokine axes.

Expression of *CCL1*, *CCR2*, and *CCL4* are of relevance with melanoma, such as this communication between immune cells and the tumor microenvironment (TME), immune evasion, and advanced-stage ulceration, respectively. Furthermore, our finding of the inverse association between *CCL2* polymorphisms and TILs aligns with the reported role of *CCL2* in shaping an immunosuppressive TME.

In summary, germline DNA from patients with cutaneous melanomas, has the potential to reveal additional insights into the potential effects of inherited CK/CKRs traits on the melanoma tumor microenvironment (TME). Other studies might confirm and extend our observations, especially if using multiple immune cell markers combined with immunofluorescence, to determine or confirm potential cell subtypes proximities and/or interaction with the tumor.

Although not part of the scope of this work, understanding tumor-immune interactions together with patient characteristics (including inherited traits) may help inform treatment options, in the future.