Special article

A functional interaction between the CCR5 and CD34 molecules expressed in hematopoietic cells can support (or even promote) the development of cancer

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ABSTRACT

Inflammation and angiogenesis are linked to the development of cancer since both can support the establishment of a tumor-prone environment. The CCR5 is a major regulatory molecule involved in inflammation. The CD34 molecule is commonly described as a hematopoietic stem cell marker, and CD34+ cells are involved in the regulation of distinct physiological processes, including angiogenesis. CCR5 participates in the development of various types of cancer, and recently, a reduced CCR5 expression was associated with low CD34+ cell counts in human cord blood. A naturally occurring genetic variant of the CCR5 gene, the so-called CCR5Δ32 polymorphism, consists of a 32 base-pair deletion in the DNA, interfering in the CCR5 protein levels on the cell surface. When in homozygosis, this variant leads to a total absence of CCR5 expression on the cell surface. In heterozygous individuals, CCR5 surface levels are reduced. Based on these key findings, we hypothesize that a functional interaction can connect CCR5 and CD34 molecules (giving rise to a “CCR5-CD34 axis”). According to this, a CCR5-CD34 interaction can potentially support the development of different types of cancer. Consequently, the lack of CCR5 in association with reduced CD34+ cell counts could indicate a protective factor against the development of cancer. It is required to characterize in detail the functional relationship between CCR5 and CD34 proteins, as well as the real influence of both molecules on the susceptibility and development of cancer at population level. If our hypothesis is confirmed, the CCR5-CD34 axis may be a potential target in the development of anti-cancer therapies.

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Background

CD34+ cells – stem cells which could turn bad

The CD34 molecule is a commonly targeted antigenic determinant used as a characteristic marker to isolate and analyze hematopoietic stem cells.1-9 This protein is expressed by all hematopoietic stem cells and its expression is lost during the cell differentiation. Therefore, mature hematopoietic cells are usually CD34 negative.1 On the other hand, this molecule is also present in a variety of mature cell types, as mesenchymal cells, muscle satellite cells, corneal keratocytes, interstitial cells, epithelial progenitors, vascular endothelial progenitors, and activated endothelial cells.10 Of note, CD34 expression has been already correlated to vasculogenic and angiogenic processes, the mesenchymal CD34+ stem cells being closely associated to vascularization.1,4-6,8,9,11 Importantly, both vasculogenic and angiogenic processes are essential to the development of cancer.12-17 Additionally, there are numerous studies describing CD34 expression in tumor cells,5,6,9,18 although the loss of this molecule has been associated with an invasive malignant phenotype.8,19 In this context, the CD34 molecule can be viewed in neoplastic cells as a dedifferentiation marker. Also, CD34 could be expressed by endothelial cells and endothelial progenitors as a potential tumor vascularization marker.

CCRS5: a chemokine receptor with multiple inflammatory functions

Chemokines are fundamental regulators of the development, differentiation, and migration of leukocytes.20-24 In addition, chemokines participate in the angiogenic process.21,25 The CC Chemokine Receptor 5 (CCRS5) is a well-known example of such molecules, being especially involved in leucocyte migration.26 Furthermore, CCR5 is well-known due to its classic role as a co-receptor molecule used by the HIV type 1 viruses.21,25,27-31 In addition, CCR5 acts in Th1 immune responses and the lack of signaling through this molecule leads to a shift in the Th2 responses.20,21 Several chemokines were described as efficient agonists of this receptor, such as RANTES (CCL5), MIP-1α (CCL3) and MIP-1β (CCL4). On the other hand, some chemokines interact less efficiently with CCR525-27,29 or are classified as CCR5 antagonists, which is the case of CCL7,25,29 Of note, the impact of CCR5 on the development of cancer is an emerging topic, with several studies suggesting that CCR5 plays an important role in the establishment of the tumor microenvironment and progression of different types of cancer. Notably, the CCR5 molecule has been related to the migration and spread of tumor cells, and therefore, to metastasis. Moreover, the presence of the receptor in neoplasms has been associated with the migration of regulatory cells and generation of an immunosuppressor tumor-prone environment. Detailed examples and other effects of CCR5 on tumorigenesis are shown in Table 1.22-24,33-44

As mentioned above, the CCR5 protein has a regulatory effect on inflammatory cells,25,27 thus it must have an enhancing function in the migration of pivotal cells for tumorigenesis, explaining at least partially its connections with the development of cancer. Importantly, the CCR5 expression was already observed in some regulatory T (Treg) cell subsets.55-56 In certain circumstances and types of cancer, Treg cells were linked with a better prognosis.51 Nevertheless, we are facing a two-edged sword, since Treg cells can also be subverted by tumor cells in order to generate a tolerogenic environment, allowing the proliferation and establishment of neoplasms.52-55 Thus, we can suppose that the absence of CCR5 in Treg cells may be a protective factor in the context of tumorigenesis.

Inflammation and the development of cancer

The role of the immune system in the development of cancer is a field of extensive debate. Even though inflammatory cells could eliminate tumor cells, inducing their death, they are also important components of the tumor microenvironment, sometimes favoring tumor growth and proliferation, also promoting neoangiogenesis.36-38 In this sense, some immune cell-derived factors, when in disbalance, can promote tumor progression. Adding to the complexity of the interactions happening in the tumor, there is a wide variety of immune cells in a neoplastic environment, including lymphocytes, macrophages, neutrophils and dendritic cells, which can produce cytokines and other mediators that enhance the tumor development.36 The immune surveillance and the individual responses to cancer therapy are also affected by different inflammatory patterns.57

CCRA32 and the CD34+ stem cell repertoire in the cord blood - a puzzling observation

Recently, a study performed by Enrich et al.58 raised intriguing results. A cohort of Spanish cord blood donors was genotyped for the CCR5Δ32 polymorphism (rs333), a genetic variant which consists of a 32 base-pair deletion in the open reading frame of the CCR5 gene. This deletion causes a premature stop-codon, which leads to the formation of a truncated protein that is not expressed on the cell surface.30,31 Heterozygous and homozygous individuals for CCR5Δ32 show respectively reduced CCR5 expression and no expression of the CCR5 molecule on the cell surface.59,60 As previously mentioned, CCR5 is an HIV-1 co-receptor and this genetic variant became widely known due to its association, when in homozygous, to resistance against HIV infection.61,62 The main objective of Enrich et al.58 was to identify the CCR5Δ32 homozygous cord blood units, which could be used as donor cells to be transplanted to HIV+ individuals. The idea was that the CCR5Δ32 homozygous cord blood units would repopulate the host with a set of cells which would not allow HIV infection, avoiding the maintenance of the virus infection, eventually leading to viral clearance. A similar approach using bone marrow CCR5Δ32 homozygous cells has been successfully applied in one patient, who remains with sustained suppression of the HIV infection.53-66 Nevertheless, among the results of Enrich et al.,58 one was surprising and unprecedented: a smaller amount of CD34+ cells was found in the samples from the CCR5Δ32 homozygous donors, as compared to both heterozygous and CCR5 wild-type homozygous. This situation represents a drawback to the main objective of the authors since a) fewer cells would be available from these donors and b)
Table 1 – Roles of CCR5 in tumorigenesis.

| Type of tumor/cell line                        | CCR5 role in tumorigenesis/key findings                                                                 | References  |
|-----------------------------------------------|---------------------------------------------------------------------------------------------------------|-------------|
| Reed Sternberg (RS) primary cells and Hodgkin Lymphoma (HL)-derived cell line | Presence of CCL5/CCR5 axis is related to tumor proliferation and microenvironment formation.            | Aldinucci et al. 43 |
| Human breast cancer cell line                 | CCR5 activation by CCL5 leads to cancer proliferation in a mTOR (mammalian Target of Rapamycin) dependent manner. | Murooka et al. 41 |
| Cervical cancer cells                         | Greater expression of CCR5 was found in cancer tissues. With downregulation of the CCR5 gene, tumor proliferation and cell invasion were diminished. | Che et al. 44 |
| Metastasis of colon rectal cancer in liver    | CCR5 was expressed by metastatic tumor cells, lymphocytes, and myeloid cells. The probable interaction between CCR5 and its ligand CCL5 produced by T lymphocytes surrounding the tumor microenvironment promotes cell invasion and metastasis. | Halama et al. 36 |
| Metastasis of mammary carcinoma in lungs      | Regulatory cells expressing high levels of CCR5 accumulate in metastatic mammary carcinomas in mice, suggesting an immunosuppressor role of CCR5, which could favor tumor development. | Halvorsen et al. 40 |
| Melanoma in mice                              | CCR5 expression by myeloid-derived suppressor cells leads to migration to primary tumors and metastatic tissues and therefore to tumor progression. | Umansky et al. 42 |

potentially cord blood units from those CCR5Δ32 homozygous donors would present a lower reconstitution potential of the host leucocyte repertoires. In this same study,28 Enrich et al. suggested that CCR5 and its agonist MIP-1α (CCL3) play a critical role in the hematopoietic stem cell function. Specifically, MIP-1α possibly regulates cytokine-induced stem-cell proliferation and the lack of CCR5 in Δ32/Δ32 individuals might disrupt the MIP-1α signaling pathway and explain the lower CD34+ cell counts.20,58,66-68 From this unexpected result, a new point emerged: a connection between CCR5 and CD34+ cells.

Linking CD34+ cells, the CCR5+ repertoire and cancer - the hypothesis

Considering that CD34+ cells were already associated with tumor development, and taking into account that the absence of CCR5+ cells (which happens in homozygous individuals of the CCR5Δ32 variant) is linked with a lower proportion of CD34+ stem cells, it is possible to infer that a functional interaction between CCR5+ and CD34+ cells exists and that this interaction can give support to cell proliferation and expansion and perhaps, to the establishment/development of different types of cancer. Consequently, the reduced expression of CCR5, in association with low CD34+ cell counts, would protect against the establishment of a tumor microenvironment and the development of cancer (Fig. 1). Additionally, data from previous studies show that the CCR5 agonist MIP-1α has a direct effect on CD34+ cells,32,58,67,68 suggesting a functional connection between CCR5 and CD34. Thereafter, this functional correlation between CCR5 and the CD34+ cells will be referred to as the “CCR5-CD34 axis”. Importantly, our hypothesis mainly addresses the role of CCR5 in the migration of inflammatory cells to the tumor environment (CCR5 as a mediator of inflammation). However, as previously mentioned, CCR5 and its ligands play an important role in Treg cell recruitment and migration of tumor cells. These different roles of CCR5 in tumorigenesis needed to be considered in our hypothesis. Taken together, an association between CCR5 and CD34 may support tumorigenesis.

Consequences of the hypothesis

The unravelling of the mechanisms of tumorigenesis is crucial for the development of new drugs and effective treatments against different types of cancer. Here we hypothesize on the existence of a not yet described interaction between two important molecules of the immune system that may act together as tumorigenesis promoters. Potentially, the CCR5-CD34 axis could be a new target for cancer treatments, pharmacologically, or as part of gene therapy strategies. However, prior to this, the interactions between CCR5 and CD34 need to be characterized at both cellular and functional levels, and their real importance in the tumor biology needs to be understood in detail.

In general, the lack of CCR5 observed in homozygous individuals of the CCR5Δ32 variant is considered not to be associated with any severe essential physiological alterations. Although it is generally accepted that homozygous individuals of this variant have no severe immunological or clinical deficiencies,20,69 exacerbated inflammatory responses have already been reported in association with the CCR5Δ32 variant.70 Interestingly, a recent report has linked CCR5 absence with defective bone development.71 Thus, it is crucial to consider that CCR5 mediates different immune functions and it is possible that its absence promotes changes of difficult detection, but with medical significance in a specific environment or context. For example, homozygous individuals of CCR5Δ32 are more susceptible to develop symptomatic West Nile virus infection.72,73 Nevertheless, CCR5Δ32 is a pleiotropic variant, promoting different outcomes in different situations.74 Of note, especially considering the recent alleged report of CCR5 editing in humans, the pros and cons of the absence of CCR5 were addressed in a recent publication by our group.75 If our hypothesis is confirmed, the
physiological significance of the absence of CCR5 needs to be reviewed. Although the chemokine-ligand system is robust and redundant,\textsuperscript{76} which ensures its functioning in the absence of some specific chemokine or chemokine receptor, alterations in such a balanced system may have a significant impact on other diseases (in a favorable or unfavorable way). Looking at the hypothesis described here, the lack of CCR5 could be considered a protective factor against the development of cancer.

**Testing our hypothesis**

We suggest the following initiatives to test our hypothesis:

- First, it is necessary to investigate the CD34\(^+\) cell counts in individuals with different CCR5\(\Delta32\) genotypes in different populations with the aim of confirming the correlation between the CCR5 expression and CD34\(^+\) cell counts.
- Second, functional studies must be performed to evaluate the potential interactions between CCR5 and CD34\(^+\) cells, helping to understand the biological significance of the CCR5-CD34 axis and identify in which contexts such interactions occur.
- Third, it is necessary to evaluate the influence of the lack of CCR5 and the reduced CD34\(^+\) cell counts (separately and in association) on the development of cancer at a populational level, as well as in vitro strategies.
- Fourth, it would be interesting to evaluate tumor growth in CCR5 and CD34 knockout animals and controls, aiming to compare the tumor progression between the groups.
- Finally, we suggest investigating the frequency of CCR5\(\Delta32\) and the cancer incidence at the populational level (in different geographic regions), in order to verify the correlation between these two data sets.

**Conclusion and perspectives**

Each cancer type comprises specific cellular and molecular environments. However, migration of inflammatory cells to the tumor site and angiogenesis are processes found in different types of solid tumors. Thus, if the influence of the CCR5-CD34 axis on the development of cancer is confirmed, it is possible that both molecules become targets for the development of new therapeutics against tumors. In addition, pharmacological strategies focusing on modulating both...
molecules together may be quite promising. Currently, the use of CCR5 blockers is already being suggested for the treatment of different pathologies, besides HIV infection, including cancer.23,24,36-38,77 The above-mentioned scenario shows that our hypothesis deserves to be investigated, once (I) it will contribute to the understanding of the factors that support the establishment of the tumor microenvironment and modify the susceptibility to the development of cancer and (II) it has the potential to reveal important implications for cancer treatment.

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Meeting of ethical standards

We declare that this study was developed according to all rules and meeting all ethical standards.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

[1] Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275(5302):964–6.
[2] Blume R, Rempel E, Mantz L, Saeed BR, Wang W, Raffel S, et al. The molecular signature of AML with increased ALDH activity suggests a stem cell origin. Leuk Lymphoma. 2018;59(9):2201–10.
[3] Civin CI, Gore SD. Antigenic analysis of hematopoiesis: a review. J Hematother. 1993;2(2):137–44.
[4] Cohen KS, Cheng S, Larson MG, Cupples LA, McCabe EL, Wang YA, et al. Circulating CD34+ progenitor cell frequency is associated with clinical and genetic factors. Blood. 2013;121(8):e50–6.
[5] Cui S, Han H, Sakata A, Hanada T, Liu T, Takai S, et al. Enhanced CD34 expression of sinusoid-like vascular endothelial cells in hepatocellular carcinoma. Pathol Int. 1996;46(10):751–6.
[6] Cummings TJ, Burchette JI, McLendon RE. CD34 and dural fibroblasts: the relationship to solitary fibrous tumor and meningioma. Acta Neuropathol. 2001;102(4):349–54.
[7] Dao MA, Nolta JA. CD34: to select or not to select? That is the question. Leukemia. 2000;14(5):773–6.
[8] Chauhan H, Abraham A, Phillips JR, Pringle JH, Walker RA, Jones JL. There is more than one kind of myofibroblast: analysis of CD34 expression in benign, in situ, and invasive breast lesions. J Clin Pathol. 2003;56(4):271–6.
[9] Breza TS, Magro CM. CD34 expression in primary cutaneous malignant melanoma: apropos of a case and review of the aberrant melanoma phenotype. J Cutan Pathol. 2005;32(10):685–9.
[10] Sidney LE, Branch MJ, Dunphy SE, Dua HS, Hopkinson A. Concise review: evidence for CD34 as a common marker for diverse progenitors. Stem Cells. 2014;32(6):1380–9.
[11] Lin C-S, Ning H, Lin G, Lue TF. Is CD34 truly a negative marker for mesenchymal stromal cells? Cytotherapy. 2012;14(10):1159–63.
[12] Tomini T, Rossi F, Claudio PP. Molecular basis of angiogenesis and cancer. Oncogene. 2003;22(42):6549–56.
[13] Hoff PM, Machado KK. Role of angiogenesis in the pathogenesis of cancer. Cancer Treat Rev. 2012;38(7):825–33.
[14] Nishida N, Yano H, Nishida T, Kamura T, Kojiri M. Angiogenesis in cancer. Vasc Health Risk Manag. 2006;2(2):219–3.
[15] Arjomandnejad M, Muhammadnejad A, Haddadi M, Sherkat-Khameneh S, Rismanchi S, Amanpour S, et al. HeLa cell line xenograft tumor as a suitable cervical cancer model: growth kinetic characterization and immunohistochemistry array. Arch Iran Med. 2014;17(4):273–7.
[16] Gao J, Li Z-h, Tang W, Wu Q-n, Liu G-h, Zheng W-b. Chemokine C-C motif ligand 18 expression correlates with tumor malignancy in breast cancer. Pathologie Biologique. 2015;63(4):199–203.
[17] Radu TG, Ciurea ME, Mogoantă ŞŞ, Busuicic CJ, Grosu F, Țenovici M, et al. Papillary thyroid cancer stroma - histological and immunohistochemical study. Rom J Morphol Embryol. 2016;57 2 Suppl:801–9.
[18] Miettinen M, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. Semin Diagn Pathol. 2006;23(2):70–83.
[19] Miettinen MM, Antonescu CR, Fletcher CDM, Kim A, Lazar AJ, Quezado MM, et al. Histopathologic evaluation of atypical neurofibromatous tumors and their transformation into malignant peripheral nerve sheath tumor in patients with neurofibromatosis 1—a consensus overview. Hum Pathol. 2017;67:1–10.
[20] Abdí R, Smith RN, Makhlouf L, Najafan N, Luster AD, Auchincloss H Jr, et al. The role of CC chemokine receptor 5 (CCR5) in islet allograft rejection. Diabetes. 2002;51(8):2489–95.
[21] Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007;25(11):2739–49.
[22] de Oliveira CE, Oda JM, Losi Guembarovsky R, de Oliveira KB, Ariza CB, Neto JS, et al. CC chemokine receptor 5: the interface of host immunity and cancer. Dis Markers. 2014;2014:126954.
[23] Velasco-Velázquez M, Xolalpa W, Pestell RG. The potential to target CCL5/CCR5 in breast cancer. Expert Opin Ther Targets. 2014;18(11):1265–75.
[24] Singh SK, Mishra MK, Eltoum IA, Bae S, Lillard JW Jr, Singh R. CCR5/CCL5 axis interaction promotes migratory and invasiveness of pancreatic cancer cells. Sci Rep. 2018;8(1):1323.
[25] Alkhateb G. The biology of CCR5 and CXCR4. Curr Opin HIV AIDS. 2009;4(2):96–103.
[26] Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. Annu Rev Immunol. 2014;32:659–702.
[27] Lederman MM, Penn-Nicholson A, Cho M, Mosier D. Biology of CCR5 and its role in HIV infection and treatment. JAMA. 2006;296(7):815–26.
[28] Moore JP, Kitchen SG, Pugach P, Zack JA. The CCR5 and CXCR4 coreceptors-central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. AIDS Res Hum Retroviruses. 2004;20(1):111–26.
[29]. Jones KL, Maguire JJ, Davenport AP. Chemokine receptor CCR5: from AIDS to atherosclerosis. Br J Pharmacol. 2011;162(2):1453–69.

[30]. Barmania F, Pepper MS. C-C chemokine receptor type five (CCR5): an emerging target for the control of HIV infection. Appl Transl Genom. 2013;2:3–16.

[31]. Scrucci I, Martins E, Hartley O. CCR5: established paradigms and new frontiers for a ‘celebrity’ chemokine receptor. Cytokine. 2018;109:81–93.

[32]. de Wynter EA, Durig J, Cross MA, Heyworth CM, Testa NG. Differential response of CD34+ cells isolated from cord blood and bone marrow to MIP-1a and the expression of MIP-1a receptors on these immature cells. Stem Cells. 1998;16(5):349–56.

[33]. Maes S, Mira E, Colomer R, Montero S, Real LM, Gómez-Moutón C, et al. CCR5 expression influences the progression of human breast cancer in a p53-dependent manner. J Exp Med. 2003;198(9):1381–9.

[34]. Vaday GG, Pehel DM, Kadam PA, Lawrence DM. Expression of CCL5 (RANTES) and CCR5 in prostate cancer. Prostate. 2006;66(2):124–34.

[35]. Wang SW, Wu HH, Liu SC, Wang PC, Ou WC, Chou WY, et al. CCL5 and CCR5 interaction promotes cell motility in human osteosarcoma. PLoS One. 2012;7(4):e35101.

[36]. Halama N, Zornig I, Berthel A, Kahler H, Klupp F, Suarez-Carmona M, et al. Tumoral immune cell exploitation in colorectal cancer metastases can be targeted effectively by anti-CCR5 therapy in cancer patients. Cancer Cell. 2016;29(4):587–601.

[37]. Gao D, Cazares LH, Fish EN. CCL5-CCR5 interactions modulate metabolic events during tumor onset to promote tumorigenesis. BMC Cancer. 2017;17(1):834.

[38]. Aldinucci D, Casagrande N. Inhibition of the CCL5/CCR5 axis against the progression of gastric cancer. Int J Mol Sci. 2018;19(5):1477.

[39]. Jiao X, Velasco-Velázquez MA, Wang M, Li Z, Rui H, Peck AR, et al. CCR5 governs DNA damage repair and breast cancer stem cell expansion. Cancer Res. 2018;78(7):1657–71.

[40]. Halvorsen EC, Hamilton MJ, Young A, Wadsworth BJ, LePard NE, Lee HH, et al. Maraviroc decreases CC-mediated migration of CCR5(+)-regulatory T cells and reduces metastatic tumor growth in the lungs. Oncoclin Immunol. 2016;5(6):e1150398, http://dx.doi.org/10.1080/2162420X.2016.1150398. Published 2016 Mar 10.

[41]. Murooka TT, Rahbar R, Fish EN. CCL5 promotes proliferation of MCF-7 cells through mTOR-dependent mRNA translation. Biochem Biophys Res Commun. 2009;387(2):361–6.

[42]. Umanésky V, Blattner C, Gebhardt C, Utikal J. CCR5 in recruitment and activation of myeloid-derived suppressor cells in melanoma. Cancer Immunol Immunother. 2017;66(8):1015–23.

[43]. Aldinucci D, Lorenzon D, Cattaruzza L, Pinto A, Gloghini A, Carbone A, et al. Expression of CCR5 receptors on Reed-Sternberg cells and Hodgkin lymphoma cell lines: involvement of CCL5/Rantes in tumor cell growth and microenvironmental interactions. Int J Cancer. 2008;122(4):769–76, http://dx.doi.org/10.1002/ijc.23119.

[44]. Che LF, Shao SF, Wang LX. Downregulation of CCR5 inhibits the proliferation and invasion of cervical cancer cells and is regulated by microRNA-107. Exp Ther Med. 2016;11(2): 503–9.

[45]. Wysocki CA, Jiang Q, Panoskaltsis-Mortari A, Taylor PA, McKinnon KP, Su L, et al. Critical role for CCR5 in the function of donor CD4+CD25+ regulatory T cells during acute graft-versus-host disease. Blood. 2005;106(9):3300–7.

[46]. Yurchenko E, Tritt M, Hay V, Shevach EM, Belkaid Y, Piccirillo GA. CCR5-dependent homing of naturally occurring CD4+ regulatory T cells to sites of Leishmania major infection favors pathogen persistence. J Exp Med. 2006;203(11):2451–60.

[47]. Soler DC, Sugiyama H, Young AB, Massari JV, McCormick TS, Cooper KD. Psoriasis patients exhibit impairment of the high potency CCR5+ regulatory T cell subset. Clin Immunol. 2013;149(1):111–8.

[48]. Tan MC, Goedengebuure PS, Belt BA, Flaherty B, Sankpal N, Gillanders WE, et al. Disruption of CCR5-dependent homing of regulatory T cells inhibits tumor growth in a murine model of pancreatic cancer. J Immunol. 2009;182(3):1746–55.

[49]. Ward ST, Li KK, Hepburn E, Weston CJ, Curbishley SM, Reynolds GM, et al. The effects of CCR5 inhibition on regulatory T-cell recruitment to colorectal cancer. Br J Cancer. 2015;112(2):319–28.

[50]. de Oliveira CE, Gasparoto TH, Pinheiro CR, Amâr NG, Nogueira MR, Kaneno R, et al. CCR5-dependent homing of T regulatory cells to the tumor microenvironment contributes to skin squamous cell carcinoma development. Mol Cancer Ther. 2017;16(12):2871–80.

[51]. Mougiakakos D, Choudhury A, Llads L, Kiessling R, Johansson CC. Regulatory T cells in cancer. Adv Cancer Res. 2010;107:57–117.

[52]. Golgher D, Jones E, Powrie F, Elliott T, Gallimore A. Depletion of CD25+ regulatory cells uncovers immune responses to shared murine tumor rejection antigens. Eur J Immunol. 2002;32(11):3267–75.

[53]. Zou W. Regulatory T cells, tumour immunity and immunotherapy. Nat Rev Immunol. 2006;6(4):295–307.

[54]. Miyara M, Sakaguchi S. Natural regulatory T cells: mechanisms of suppression. Trends Mol Med. 2007;13(3):108–16.

[55]. Facciabene A, Motz GT, Coukos G. T-regulatory cells: key players in tumor immune escape and angiogenesis. Cancer Res. 2012;72(9):2162–71.

[56]. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860–7.

[57]. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140(6):883–99.

[58]. Enrich R, Vidal F, Sánchez-Gordo F, Gómez-Zumaquero JM, Balas A, Rudilla F, et al. Analysis of the Spanish CCR5-Δ32 inventory of cord blood units: lower cell counts in homozygous donors. Bone Marrow Transplant. 2018;53(6):741–8.

[59]. Wu L, Paxton WA, Kassam N, Ruffing N, Rottman JB, Sullivan N, et al. CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, in vitro. J Exp Med. 1997;185(9):1681–91.

[60]. Venkatesan S, Petrovic A, Van Ryk DJ, Locati M, Weissman D, Murphy PM. Reduced cell surface expression of CCR5 in CCR5Δ32 heterozygotes is mediated by gene dosage, rather than by receptor sequestration. J Biol Chem. 2002;277(3):2287–301.

[61]. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell. 1996;86(3):567–77.

[62]. Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, et al. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR5-5 chemokine receptor gene. Nature. 1996;382(6593):722–5.

[63]. Hütter G, Nowak D, Mossier N, Canepola S, Müssig A, Allers K, et al. Long-term control of HIV by CCR5Δ32/Δ32 stem-cell transplantation. N Engl J Med. 2009;360(7):692–8.

[64]. Allers K, Hütter G, Hofmann J, Loddenkemper C, Rieger K, Thiel E, et al. Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation. Blood. 2011;117(10):2791–9.
[65]. Brown TR. Timothy Ray Brown's continuing activism toward curing HIV. AIDS Res Hum Retroviruses. 2018;34(1):9–11.

[66]. Clements JM, Craig S, Gearing AJ, Hunter MG, Heyworth CM, Dexter TM, et al. Biological and structural properties of MIP-1α expressed in yeast. Cytokine. 1992;4(1):76–82.

[67]. Mayani H, Little MT, Dragowska W, Thornbury G, Lansdorp PM. Differential effects of the hematopoietic inhibitors MIP-1 alpha, TGF-beta, and TNF-alpha on cytokine-induced proliferation of subpopulations of CD34+ cells purified from cord blood and fetal liver. Exp Hematol. 1995;23(5):422–7.

[68]. Capmany G, Querol S, Cancelas JA, Garcia J. Short-term, serum-free, static culture of cord blood-derived CD34+ cells: effects of FLT3-L and MIP-1α on in vitro expansion of hematopoietic progenitor cells. Haematologica. 1999;84(8):675–82.

[69]. Nguyễn GT, Carrington M, Beeler JA, Dean M, Aledort LM, Blatt PM, et al. Phenotypic expressions of CCR5-Δ32/Δ32 homozygosity. J Acquir Immune Defic Syndr. 1999;22(1):75–82.

[70]. Vargas AE, Cechim G, Correa JF, Gomes PA, Macedo GS, de Medeiros RM, et al. Pros and cons of a missing chemokine receptor - Comments on “Is the European spatial distribution of the HIV-1-resistant CCR5-Δ32 allele formed by a breakdown of the pathogenesis due to the historical Roman expansion?” by Eric Faure and Manuela Royer-Carenzi (2008). Infect Genet Evol. 2009;9(4):387–9.

[71]. Xie Y, Zhan S, Ge W, Tang P. The potential risks of C-C chemokine receptor 5-edited babies in bone development. Bone Res. 2019;7:4, http://dx.doi.org/10.1038/s41413-019-0044-0. Published 2019 Jan 29.

[72]. Glass WG, McDermott DH, Lim JK, Lekhong S, Yu SF, Frank WA, et al. CCR5 deficiency increases risk of symptomatic West Nile virus infection. J Exp Med. 2006;203(1):35–40.

[73]. Lim JK, McDermott DH, Lisco A, Foster GA, Krysztof D, Follmann D, et al. CCR5 deficiency is a risk factor for early clinical manifestations of West Nile virus infection but not for viral transmission. J Infect Dis. 2010;201(2):178–85.

[74]. Li T, Shen X. Pleiotropy complicates human gene editing: CCR5Δ32 and beyond. Front Genet. 2019;10.

[75]. Ellwanger JH, Kaminski VL, Chies JA. CCR5 gene editing - Revisiting pros and cons of CCR5 absence. Infect Genet Evol. 2019;68:218–20.

[76]. Mantovani A. The chemokine system: redundancy for robust outputs. Immunol Today. 1999;20(6):254–7.

[77]. Vangelista L, Vento S. The expanding therapeutic perspective of CCR5 blockade. Front Immunol. 2018;8:1981.