

# Atypical chemokine receptor 2: a brake against Kaposi's sarcoma aggressiveness

Raffaella Bonecchi<sup>1,2,\*</sup>, Benedetta Savino<sup>1,2</sup>, Nicoletta Caronni<sup>1,2</sup>, Giuseppe Celesti<sup>1</sup>, Alberto Mantovani<sup>1,2</sup>, and Massimo Locati<sup>1,2</sup>

<sup>1</sup>Humanitas Clinical and Research Center; Rozzano, Italy; <sup>2</sup>Department of Medical Biotechnologies and Translational Medicine; Università degli Studi di Milano; Rozzano, Italy

**Keywords:** atypical chemokine receptors, chemokines, inflammation and cancer, Kaposi's sarcoma, macrophages

Inflammatory chemokines are instrumental players in cancer-related inflammation contributing to numerous steps during tumor progression. In Kaposi's sarcoma, we have found that downregulation of the atypical chemokine receptor 2 (ACKR2) by the KRAS/BRAF/ERK pathway profoundly affects the tumor microenvironment, unleashing accumulation of tumor-associated macrophages that sustains tumor growth. This discovery extends our understanding on the role of inflammatory chemokines in tumor biology and provides rationale for their therapeutic targeting.

Cancer-related inflammation fuels fundamental aspects of tumor growth and is therefore listed among the contemporary hallmarks of cancer.<sup>1</sup> Neoplasia-associated inflammation supplies soluble growth and proangiogenic factors, produces extracellular matrix-modifying enzymes and, further, can even directly promote mutagenic events and can foster activation of epithelial-mesenchymal transition (EMT).<sup>2</sup> A noteworthy aside, chronic inflammation is a predisposing factor for the inception of many tumors but it is also induced by the developing neoplasm itself, underlining that inflammatory conditions are often required for tumor growth and malignant disease progression. Chemokines and their receptors are highly expressed in many tumors due to inactivation of tumor suppressor genes or constitutive activation of oncogenes, either of which may directly target differential chemokine expression or indirectly upregulate chemokine signaling axis by deregulated expression of transcription factors.<sup>3</sup> The chemokine system plays a central role in orchestrating cancer-related inflammation through recruitment and activation of immune cells within the tumor microenvironment.

In the chemokine system a small subgroup of atypical chemokine receptors (ACKR) has been identified. These receptors do not signal through G proteins and lack chemotactic activity but share the

important function of chemokine scavenging, thus controlling local chemokine concentrations. ACKR2 is mainly expressed by endothelial cells of lymphatic vessels and is able to bind and drive to degradation thus negatively regulating nearly all inflammatory C—C motif chemokines.<sup>4</sup> Evidence *in vivo* have clearly revealed its non-redundant role in resolving inflammation, regulating inflammatory monocyte mobilization and recruitment,<sup>5</sup> and, in the suppression of inflammation-driven tumor development.<sup>6,7</sup>

It has been long known that ACKR2 is expressed by vascular tumors and by Kaposi's sarcoma (KS) lesions,<sup>8</sup> but the role of ACKR2 in the biology of these tumors was obscure. KS is a complex tumor that arises from infection of lymphatic endothelial cells by KS herpesvirus/human herpesvirus-8 (KSHV/HHV8). The infection itself is not able to induce KS and indeed cancer occurs predominantly in immunocompromised individuals, in inflammatory sites or in scarring tissues (Koebner phenomenon) and in patients with immune reconstitution inflammatory syndrome (IRIS).

In order to promote KS growth, HHV8 hijacks the chemokine system via encoding an oncogenic and constitutively active chemokine receptor and chemokines that comprise a strategy to subvert and divert effective antiviral and

antitumor immunity. In addition, HHV8-infected endothelial cells display increased production of several chemokines, including CCL2, CCL5, CXCL8, and CXCL16 that promote the infiltration of tumor by inflammatory cells.

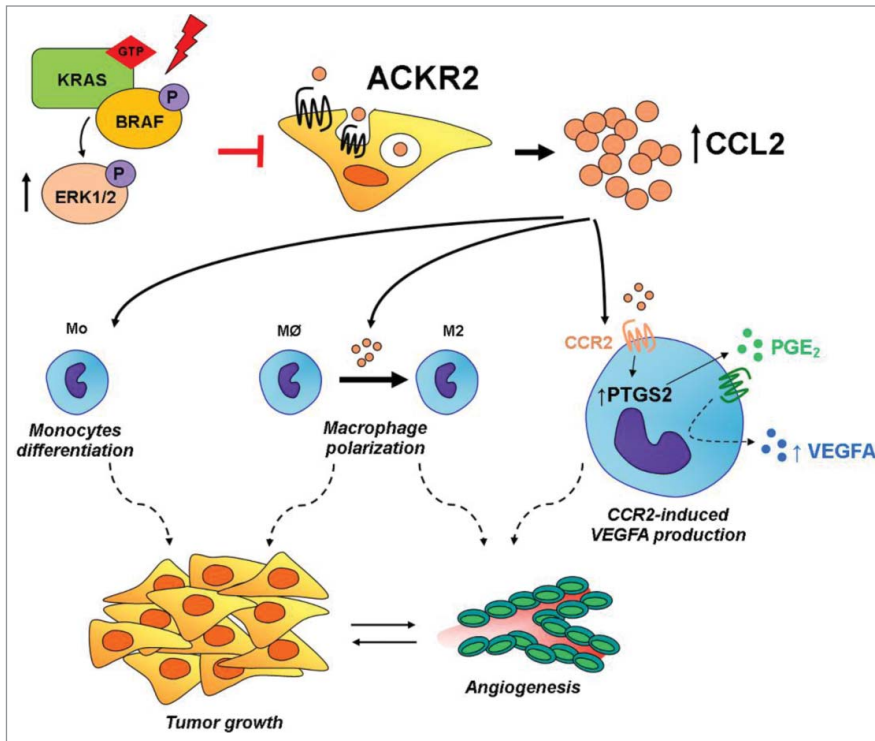
In our recent article<sup>9</sup> we found an additional mechanism that acts on the chemokine system allowing KS to increase macrophage tumor-infiltration to promote its aggressiveness. In KS lesions from 2 different cohorts of patients we observed that the expression of ACKR2 is downregulated in more aggressive patient tumors. Using a human KS cell line, we subsequently demonstrated that tumors lacking ACKR2 expression grew faster and reached larger sizes. Analysis of tumors lacking ACKR2 revealed increased tumor-associated macrophages (TAM) with an alternative M2-like phenotype and increased angiogenesis as compared to tumors expressing the atypical chemokine receptor. We also found evidence for a negative correlation between ACKR2 expression and the level of infiltrating M2-like macrophages in human KS lesions, and further demonstrated the functional relevance of monocyte recruitment for KS growth by performing targeted depletion experiments.

It is well known that tumor-associated macrophages (TAM) exert many tumor-supporting functions and their depletion

\*Correspondence to: Raffaella Bonecchi; Email: raffaella.bonecchi@humanitasresearch.it

Submitted: 07/23/2014; Accepted: 07/28/2014

<http://dx.doi.org/10.4161/21624011.2014.955337>



**Figure 1.** Mechanism by which the chemokine scavenger ACKR2 is downregulated in Kaposi's sarcoma and its effects on tumor growth. Oncogenic mutations in the KRAS pathway downregulate the expression of atypical chemokine receptor 2 (ACKR2) in Kaposi's sarcoma (KS) spindle cells, unleashing elevated levels of the inflammatory chemokine CCL2 to recruit monocytes and drive their differentiation toward M2-like macrophages. CCL2 induces expression of prostaglandin-endoperoxide synthase 2 (PTGS2) that increases the production of vascular derived endothelial growth factor A (VEGFA) through the prostaglandin E2 (PGE<sub>2</sub>) pathway supporting tumor angiogenesis.

is a promising therapeutically approach. Despite KS has been recognized to have a strong macrophage infiltrate, in our study we demonstrated for the first time that TAM infiltration is essential for enhancing KS growth.

As mentioned before ACKR2 is able to facilitate the degradation of almost all inflammatory chemokines. Having found increased levels of CCL2 in KS lacking ACKR2, and due to the fact that CCL2 is the main chemokine attracting macrophages in the tumor context, we focused our attention on the CCL2/CCR2 axis.<sup>9</sup> First, we demonstrated that coinjection of KS cells with macrophages promotes tumor growth only when they express CCR2. Next, we demonstrated that

CCR2 is required for increased macrophage-mediated production of vascular endothelial derived growth factor A (VEGFA) in response to stimulation with KS supernatant *in vitro*. We also determined that the increased production of VEGFA is dependent on prostaglandin-endoperoxide synthase 2 (PTGS2) activity, consistently with previous publications indicating that CCR2 increases the release of this angiogenetic factor through an autocrine loop passing through upregulation of PTGS2 and production of prostaglandin E2 (PGE<sub>2</sub>).

The final point we addressed is the mechanism by which ACKR2 is downregulated by the tumor. We focused our attention on the KRAS pathway because

of prior evidence that ACKR2 can be downregulated by the KRAS oncogene<sup>10</sup> and since oncogenic mutations or amplifications interfering with the activity of this pathway are frequently observed in late-nodular KS lesions and angiosarcomas. We found that the KS cell line we used harbors an activating mutation in the BRAF gene and that ACKR2 expression is under control of this signaling pathway. Furthermore analysis of human KS lesions indicated that ACKR2 expression negatively correlate with the expression of the phosphorylated form of ERK, the downstream target of the KRAS pathway.

Taken together, our data indicate that during KS progression oncogenic events activating the ERK1/2 pathway induce ACKR2 downregulation in sarcoma cells (Fig. 1), an instrumental response permitting the tumor to unleash a chemokine-dependent recruitment of monocytes. We have outlined, in particular, an important role for CCL2 not only for sustaining monocyte recruitment but also for driving macrophage polarization toward a M2-like proangiogenic phenotype, providing a rationale for targeting these inflammatory components as a complementary strategy to treat KS.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Funding

Research activities in the lab are supported by Ministero dell'Istruzione dell'Università e della Ricerca (FIRB projects), Ministero della Salute (Ricerca Finalizzata), the Italian Association for Cancer Research (AIRC), and the European Community's Seventh Framework Program [FP7-2007-2013] under grant agreement HEALTH-F4-2011-281608 (TIMER).

#### References

- Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008; 454:436-44; PMID: 18650914; <http://dx.doi.org/10.1038/nature0720>
- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144:646-74; PMID:21376230; <http://dx.doi.org/10.1016/j.cell.2011.02.013>
- Mantovani A, Savino B, Locati M, Zampataro L, Allavena P, Bonecchi R. The chemokine system in cancer biology and therapy. *Cytokine Growth Factor Rev*

- 2010; 21:27-39; PMID:20004131; <http://dx.doi.org/10.1016/j.cytogfr.2009.11.007>
4. Mantovani A, Bonocchi R, Locati M. Tuning inflammation and immunity by chemokine sequestration: decoys and more. *Nat Rev Immunol* 2006; 6:907-18; PMID:17124512; <http://dx.doi.org/10.1038/nri1964>
  5. Savino B, Castor MG, Caronni N, Sarukhan A, Anselmo A, Buracchi C, Benvenuti F, Pinho V, Teixeira MM, Mantovani A, et al. Control of murine Ly6C (high) monocyte traffic and immunosuppressive activities by atypical chemokine receptor D6. *Blood* 2012; 119:5250-60; PMID:22504926; <http://dx.doi.org/10.1182/blood-2011-10-388082>
  6. Nibbs RJ, Gilchrist DS, King V, Ferra A, Farrow S, Hunter KD, Graham GJ. The atypical chemokine receptor D6 suppresses the development of chemically induced skin tumors. *J Clin Invest* 2007; 117:1884-92; PMID:17607362; <http://dx.doi.org/10.1172/JCI30068>
  7. Vetrano S, Borroni EM, Sarukhan A, Savino B, Bonocchi R, Correale C, Arena V, Fantini M, Roncalli M, Malesci A, et al. The lymphatic system controls intestinal inflammation and inflammation-associated colon cancer through the chemokine decoy receptor D6. *Gut* 2010; 59:197-206; PMID:19846409; <http://dx.doi.org/10.1136/gut.2009.183772>
  8. Nibbs RJ, Kriehuber E, Ponath PD, Parent D, Qin S, Campbell JD, Henderson A, Kerjaschki D, Maurer D, Graham GJ, et al. The beta-chemokine receptor D6 is expressed by lymphatic endothelium and a subset of vascular tumors. *Am J Pathol* 2001; 158:867-77; PMID:11238036; [http://dx.doi.org/10.1016/S0002-9440\(10\)64035-7](http://dx.doi.org/10.1016/S0002-9440(10)64035-7)
  9. Savino B, Caronni N, Anselmo A, Pasqualini F, Borroni EM, Basso G, Celesti G, Laghi L, Tournalaki A, Boneschi V, et al. ERK-dependent downregulation of the atypical chemokine receptor D6 drives tumor aggressiveness in Kaposi sarcoma. *Cancer Immunol Res* 2014; 2:679-89; PMID:24844911; <http://dx.doi.org/10.1158/2326-6066.CIR-13-0202>
  10. Lee S, Kang J, Cho M, Seo E, Choi H, Kim E, Kim J, Kim H, Kang GY, Kim KP, et al. Profiling of transcripts and proteins modulated by K-ras oncogene in the lung tissues of K-ras transgenic mice by omics approaches. *Int J Oncol* 2009; 34:161-72; PMID:19082487