KRAS Oncogenic Signaling Extends beyond Cancer Cells to Orchestrate the Microenvironment

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Abstract

KRAS is one of the most frequently mutated oncogenes in cancer, being a potent initiator of tumorigenesis, a strong inducer of malignancy, and a predictive biomarker of response to therapy. Despite the large investment to understand the effects of KRAS activation in cancer cells, pharmacologic targeting of KRAS or its downstream effectors has not yet been successful at the clinical level. Recent studies are now describing new mechanisms of KRAS-induced tumorigenesis by analyzing its effects on the components of the tumor microenvironment. These studies revealed that the activation of KRAS on cancer cells extends to the surrounding microenvironment, affecting the properties and functions of its constituents. Herein, we discuss the most emergent perspectives on the relationship between KRAS-mutant cancer cells and their microenvironment components. Cancer Res; 78(1); 7–14. ©2017 AACR.

Introduction

Cancer is a major health concern and a leading cause of death, surpassed only by heart diseases, an order that is expected to reverse in the foreseeable future (1). Cancer prognosis is intimately linked to disease stage. At the most advanced stages, when metastases occur, prognosis is generally aggravated, and treatments are most likely to fail (2).

In 2000, Hanahan and Weinberg established the fundamental pillars of cancer as the resistance against cell death, sustainment of proliferative signaling, evasion of growth suppressors, activation of invasion and metastasis, replicative immortality, and angiogenesis (3). Genomic instability and mutations were pointed out of invasion and metastasis, replicative immortality, and angiogenesis (3). Genomic instability and mutations were pointed out as the main mechanisms underlying the acquisition of these hallmark features (4). Nevertheless, besides inflammatory and immune cells, other resident or recruited stromal cells, such as fibroblasts, endothelial cells, adipocytes, pericytes, and components of the extracellular matrix (ECM), actively contribute to the induction of these hallmark capabilities (5) through direct interaction or diffused secreted factors. In fact, in advanced cancer stages, the microenvironment is considered as a central modulator of features such as cancer cell invasion, intravasation, and extravasation from surrounding blood vessels, and capacity to home and colonize new niches, forming metastases (6). The microenvironment induces transient changes in gene profile, with impact on the metastatic features of cancer cells by promoting metabolic, proliferative, migratory, or differentiation shifts (7–9). Notably, the tumor microenvironment also participates in the tight regulation of quiescent metastases, as alterations on stromal cells and ECM components inhibit cancer cell elimination by the immune system and promote escape from immune surveillance, promoting awakening from dormancy (10). However, at early stages of cancer development, the microenvironment may exert a negative pressure, which, through interaction with cancer cells, is frequently overcome, shifting the balance from an antitumorigenic to a protumorigenic microenvironment (11). As such, cancer cells and tumor microenvironment are seen as interactive units that evolve together in time rather than separate entities (12). In this scenario, cancer cell alterations acquire a key role by conferring a fitness advantage in a given environment or by providing tools to modulate the surrounding repressive environment.

One of the most frequently mutated oncogenes in cancer is KRAS. KRAS gene encodes a small GTPase, which cycles between GDP and GTP-bound states as a consequence of the stimulation of endothelial cell signals or the modulation of cancer-associated inflammatory/immune cells, which translate into...
critical events to sustain tumor growth, and ultimately lead to the invasion and migration of cancer cells. Although discussing the effect of KRAS-mutant cancer cells on tumor microenvironment components in separate sections, it is important to keep in mind that all the components are intimately connected and regulate each other’s properties (22). As such, by affecting one of the components, KRAS-mutant cancer cells are likely affecting the entire microenvironment in a snowball-like effect. This comprehensive dissection might enlighten the way to properly deal with KRAS signals so as to prevent cancer cells from disseminating through the organism.

Immunomodulatory Effects of KRAS-Mutant Cancer Cells

Immune cells, such as CD4+ T cells, regulatory T lymphocytes (Treg), B cells, CD8+ T lymphocytes (also known as cytotoxic T lymphocytes), TH17 cells (TH17), natural killer (NK) cells, macrophages, myeloid-derived suppressor cells (MDSC), neutrophils, dendritic cells, mast cells, and platelets, often infiltrate the tumor stroma. Whereas cells with professional cytotoxic activity, as CD8+ T lymphocytes and NK cells, generally exert antitumorigenic activities, MDSCs, mast cells, and platelets are mainly protumorigenic. Notably, the remaining cell types, although exhibiting antitumorigenic properties, may, upon microenvironment subversion, acquire mitogenic, promitotic, protumorigenic, or prosurvival properties (5).

Usually, tumors are densely infiltrated by immune cells, even those that are not epidemiologically related to inflammation (23). In these situations, escape from their negative regulatory action is paramount for cancer establishment and maintenance (24). The interplay between cancer and immune cells seems to play a key role in this process, and several studies have now pointed out the pivotal role of KRAS activation in mediating this cross-talk, promoting the switch from an antitumorigenic to a protumorigenic response. In fact, regulation of tumor-associated immune responses by KRAS-mutant cancer cells has been reported to occur at the level of recruitment, of tumor-associated immune responses by KRAS-mutant cancer cells and myeloid cells, in particular macrophages and neutrophils in tumor stroma (25). Little evidence can be found in the literature regarding the association of KRAS-mutant cancer cells and myeloid cells, in particular macrophages and neutrophils infiltration.

In mouse models, it has been shown that inflammation potentiates the growth and progression of KRAS-mutant lesions (26, 27). These growth advantages result from the combination of highly proliferative potential of KRAS-mutant cancer cells with their capacity to modulate the nature of the inflammatory response. Accordingly, activation of KRAS in mouse pancreatic acinar cells triggered a local inflammatory response enriched on macrophage infiltration, promoting progression toward a more advanced duct-like phenotype. This activation occurred as a result of KRAS-induced upregulation of intercellular adhesion molecule expression in acinar cells, which served as a chemoattractant to macrophages. Counterintuitively, it specifically promoted the recruitment of the inflammatory, antitumorigenic M1-like macrophages. However, secretion of proinflammatory cytokines and proteases, such as TNF and matrix metalloprotease-9 (MMP-9), respectively, by M1 macrophages cooperated with KRAS activation to promote progression from acinar to ductal metaplasia, accelerating the pathogenesis of pancreatic cancers (Fig. 1A; refs. 28–30).

Other studies have shown that KRAS may also be responsible for determining the specificities of the immune responses among cancer subtypes. This may be the case of lung tumors in which the analysis of the host inflammatory immune response in small-cell lung cancers and adenocarcinomas revealed that adenocarcinomas were more densely infiltrated by cells from the myeloid lineage (macrophages, neutrophils, and eosinophils), whereas small-cell lung cancers predominantly showed T-cell infiltration (31). Knowing that KRAS mutations are more frequently found in adenocarcinomas, these results suggest an association between KRAS signaling activation and inflammatory immune cell infiltration. Nevertheless, it is important to take into consideration that not all inflammatory immune cells present within the tumor tissue may be a direct consequence of the presence of mutant KRAS. One study (32) has demonstrated that macrophages were preferentially localized on the periphery of the tumor, whereas neutrophils infiltrated the tumor stroma. A more detailed analysis revealed that, in fact, KRAS-mutant tumor cells secreted high levels of neutrophil chemokines, as CXCL2, CXCL5, and CXCL1, whereas small-cell lung cancers predominantly showed T-cell infiltration (33). These data establish a direct link between KRAS activation and neutrophil recruitment in lung tumorigenesis and suggest that macrophage recruitment may not be directly associated with the presence of a KRAS mutation.

Besides the direct protumorigenic effects that myeloid cell–derived secreted factors have on cancer cells, the recruitment of specific populations by mutant KRAS cancer cells may also indirectly impact on tumor growth and development through the capacity that these cells have to modulate the response and activation T lymphocytes (25). This was demonstrated in a KRAS/p53 mouse model of pancreatic cancer, in which granulocyte macrophage colony-stimulating factor (GM-CSF) derived from KRAS-mutant cells promoted the expansion of MDSCs. On their turn, MDSCs suppressed the antitumor activity of CD8+ cytotoxic T cells (Fig. 1C; ref. 33). The same effect is likely to occur in the colorectal cancer model as GM-CSF was shown to be upregulated by mutant KRAS (34).

Despite the topic being still very unexplored, altogether, the data described above for KRAS activation a role on the modulation of tumor-associated immune responses. Because tumor-associated myeloid cells are known to interfere with virtually all of the current anticancer treatments, and myeloid cells targeting is becoming an appealing therapeutic strategy (35), further studies are needed to better understand how KRAS-mutant cancer cells affect these immune cell populations.

Direct Regulation of T-cell Populations by Mutant KRAS and Its Implication on Immune Evasion

Paradoxically, it has long been known that cancer cells harboring KRAS mutations, although escaping elimination by the
immunocytes, produce high amounts of the mutant neoantigens with potential to be recognized by CD8\(^+\) and CD4\(^+\) T cells. In accordance, several studies have shown that it is possible to generate specific antitumoral T-lymphocyte responses upon adoptive cell transfer of T cells previously challenged with different KRAS-mutant epitopes (36–40). As such, attenuation or even suppression of the adaptive immune response against KRAS-mutant tumor cells is therefore an essential requirement for them to escape the tight immune control, survive, and develop into a cancer. Several mechanisms through which KRAS-mutant cancer cells mitigate the antitumoral T-cell response have been described. It was reported that KRAS activation mediates immune evasion by downregulating MHC class I antigen presentation at the cell surface, therefore decreasing cancer cell recognition by CD8\(^+\) cytotoxic T cells (Fig. 1D). This effect is mediated through the modulation of the processing machinery of MHC class I proteins rather than affected by mRNA expression levels (41, 42). Accordingly, knockdown of mutant KRAS in a highly immunogenic mouse cell line enhanced T-cell–mediated tumor cell clearance, leading to a decrease in tumor burden, an increase in the lag time, and induction of tumor regression. This effect was attributed to an increase in cell surface expression of H-2K\(_d\) MHC class I protein, and to an increase in the expression and secretion of the immune-stimulatory cytokine IL18, upon KRAS silencing (43). In addition, the capacity of KRAS-mutant cells to convert CD4\(^+\) Th cells into functional Tregs was also described as another mechanism by which mutant KRAS suppresses T-cell activation, promoting a tolerogenic microenvironment. At the molecular level, activation of MEK\(_{-}\)ERK\(_{-}\)AP1 pathway by mutant KRAS induced expression and secretion of the suppressive cytokines IL10 and TGF\(\beta\) by cancer cells, identified as the main promoters for Treg induction of differentiation (Fig. 1E; ref. 38). Supporting a functional role for Tregs in cancer, these immunosuppressive cells have been described to infiltrate the stroma of several solid and hematologic tumors.

Figure 1. Paracrine effects of KRAS-mutant cancer cells on tumor microenvironment components. Depending on the tumor model, activation of KRAS in cancer cells has been shown to promote cancer progression by influencing the properties of several tumor microenvironment components. KRAS-mutant cancer cells secrete molecules that will promote the recruitment of M1 macrophages (A) and neutrophils (B). The induction of a less reactive and more tolerogenic environment is achieved through recruitment of MDSCs (C), inhibition of CD8\(^+\) cytotoxic lymphocyte activation (D), and induction of Treg differentiation (E). Th17 recruitment (F), fibroblast activation (G), endothelial cell recruitment, and blood vessel formation (H) as well as ECM remodeling (I) represent other microenvironment alterations led by mutant KRAS cells.
cancers (44), and to act as important partners in the tumorigenic process driven by mutant KRAS (45). Besides Treg induction, activation of KRAS in mouse lung and pancreatic epithelium was also reported to increase the number of Th17 cells, a proinflammatory subset of T cells, as well as of γδ TCR+ inflammatory cells, which were shown to accelerate tumor formation (46–48). These cells produce high levels of IL17 cytokine, which in turn promote tumor cell proliferation, angiogenesis, production of proinflammatory cytokines (IL6, CXCL2, CCL2, ARG1, and CSF3), metalloproteases (MMP-7 and MMP-12), and stimulate the recruitment of MDSCs (47). An increase in IL17 expression was also observed in KRAS-mutant colorectal cancers (34). In the pancreatic cancer model, it was also shown that, besides recruiting IL17-producing cells, KRAS activation induces the expression of IL17A receptor in the neoplastic cells, establishing a hematopoietic-to-epithelial IL17 signaling axis important for the initiation and progression of pancreatic intraepithelial lesions (Fig. 1F; ref. 48).

In addition, a comparison between the immune cell content of KRAS- and EGFR-mutant lung adenocarcinomas revealed that KRAS-mutant tumors exhibit an enhanced infiltration of CD8+ T cells, Tregs, and IL17A-producing lymphocytes and reduced NK cells (31). Although not establishing a causal relationship, this work further supports the role of KRAS in controlling the immune cell landscape of tumors in the human context and suggests that, even within the same tumor subtype, cancer cell mutational profile may strongly impact on the immune landscape.

**Clinical Implications of the Immunomodulatory Capacity of KRAS-Mutant Cancer Cells**

The studies referred above illustrate the effect of KRAS mutations in the construction of a favorable immune microenvironment that supports escape from immunosurveillance and promotes disease progression. At the clinical viewpoint, these observations are of critical importance as they pave the way to better understand in which way KRAS activation affects the response to immunotherapeutic approaches. For instance, this would be relevant in the case of current antibody-mediated blockage of the signaling between programmed cell death protein 1 (PD-1), an immune checkpoint receptor upregulated in activated T cells to induce immune tolerance, and its ligand, the programmed cell death ligand 1 (PD-L1), frequently overexpressed in tumor cells (49).

Recent works performed in lung cancer revealed that PD-L1 expression is associated with KRAS mutations, smoking, and wild-type EGFR, and that PD-L1 upregulation occurs through KRAS-mediated ERK signaling (50, 51). Nevertheless, some authors revealed that the association of KRAS and PD-L1 expression levels is dependent on the association with other lung cancer–associated gene mutations, such as TP53 and STK11/LKB1 (52–54). In accordance, the highest levels of PD-L1 as well as elevated PD-L1/CD8+ cell ratio was found in KRAS and TP53 comutated lung tumors. In addition, TP53, KRAS, and specially TP53/KRAS comutated patients were the ones that treated with pemetrexed, a humanized antibody targeting PD-1, showed a significant prolonged progression-free survival compared with wild-type patients (52). On its turn, inactivation of STK11/LKB1 in a KRAS-mutant background was associated with lower levels of PD-L1 expression (53). Corroborating this work, another group found, in a mouse model of KRAS-driven NSCLC, that the downregulation of the tumor suppressor STK11/LKB1 resulted in accumulation of neutrophils with T-cell–suppressive effects and reduced PD-L1 expression (54). In addition, anti–PD-1 therapy enhanced the effects of radiotherapy in radiation-naïve KRAS-mutant mouse tumors. However, this synergistic effect was lost upon additional inactivation of STK11 (55), further supporting that retention of normal STK11/LKB1 protein is essential for the establishment of a mutant KRAS-driven immunosuppressive environment.

The associations between KRAS activation and the expression of PD-L1 and PD-1 were also studied in pancreatic and colorectal cancers, the other two cancer models in which KRAS mutations are highly prevalent, although the data available are still scarce. In the case of pancreatic cancer, KRAS activation was shown to be associated with increased PD-1 expression (56). In contrast to lung and pancreatic cancers, in colorectal cancer, the available data show that KRAS mutations predict low PD-L1 expression and poor immune infiltration (57–59).

The positive associations observed between KRAS mutations and the expression of PD-1/PD-L1 immunosuppressive axis in lung and pancreatic cancers indicate that these therapeutic strategies are likely to impair progression of these KRAS-mutant tumors. These results represent a good example of how to target this group of tumors by abrogating their interaction with microenvironment components. In colorectal cancer, however, the presence of KRAS mutations may be indicative of lack of anti-PD-1/PD-L1 therapy efficacy.

**Modulation of the Properties of Cancer-Associated Fibroblasts by Mutant KRAS Cancer Cells**

Fibroblasts are one of the most abundant cell types present within the tumor stroma, and, given their plasticity, may become promptly activated through the interaction with tumor cells. Cancer-associated fibroblasts are thereby able to promote tumor initiation and progression as they function as master promoters of epithelial-to-mesenchymal transition, cancer cell migration, invasion and metastasis as well as regulators of ECM remodeling and dynamics, and angiogenesis (60, 61). As such, the capacity of cancer cells to modulate the properties of fibroblasts is predicted to have a major impact on the characteristics of the tumor microenvironment. Studies using pancreatic cancer models have been pointing out a role for KRAS in mediating the activation of fibroblasts through the Hedgehog (Hh) signaling. The deregulation of Hh signaling is long known to have an impact in cancer (62), but the mechanisms through which it happens remained unresolved. The Hh pathway, through the Sonic Hedgehog (Shh) protein, is known to promote myofibroblast expansion (63, 64), and its depletion was shown to reduce pancreatic ductal carcinoma (PDAC) stroma (65). Supporting these observations, another group showed that when Shh was activated in pancreatic epithelia, transgenic mice would develop undifferentiated carcinoma (66). However, these mice did not completely mimic human pancreatic carcinogenesis unless Ras was simultaneously activated. This was one of the first suggestions that RAS and SHH cooperated in the promotion of pancreatic carcinoma development. Still within pancreatic models, Ji and colleagues hypothesized that oncogenic KRAS would promote tumorigenesis by activating the Shh pathway (67). In accordance, they identified an increase of GLI1 activity downstream of the RAF/MEK/MAPK pathway.
pathway. GLI1 was later found to bind to IL6 promoter in fibroblasts of the tumor microenvironment, increasing IL6 expression. On its turn, fibroblasts-derived IL6 was shown to be a modulator of STAT3, a transcriptional factor required for the development of premalignant lesions and the progression into pancreatic cancer (Fig. 1G; ref. 68). These studies hinted at a role of VEGF in the progression of pancreatic cancer (Fig. 1G; ref. 68). These studies hinted at a role of VEGF in the progression of pancreatic cancer (Fig. 1G; ref. 68). These studies hinted at a role of VEGF in the progression of pancreatic cancer (Fig. 1G; ref. 68).

The impact of KRAS on the regulation of the most potent angiogenic factor VEGF has been extensively studied in different models (74–76). Notably, in pancreatic cancer cells, silencing of mutant KRAS in two different cell lines reduced the angiogenic potential. Different mechanisms underlying the observed effect were described. Panc-1 cells increased the expression level of thrombospondin-1, an endogenous inhibitor of angiogenesis, whereas MiaPaca-2 cells decreased the production of VEGF (Fig. 1H; ref. 77). Furthermore, using mouse fibroblasts transfected with oncogenic KRAS, the impact on angiogenesis through the regulation of VEGF is distinct, depending on the mutation. Transfectants with codon 13 mutation had higher VEGF expression and secretion than the ones with codon 12 mutation, which was then translated in a more complex vascular structure in subcutaneous tumors. It is noteworthy this difference was a consequence of VEGF differential transcriptional activity orchestrated by RAS–RAF–ERK–AP2/Spi1 signaling, independent of hypoxia-dependent elements (78).

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Modulation of ECM Composition and Structure by Mutant KRAS Cancer Cells

Although frequently underestimated, the ECM is one of the most abundant elements at the tumor microenvironment. It consists of a highly dynamic and complex network of macromolecules, acting as a reservoir of numerous bioactive domains and arrested growth factors involved in the regulation of several cancer cell activities (86, 87).

The most recent literature supports the existence of discrete connections between KRAS activation and the modulation of the ECM, through induced secretion of both matrix components and matrix remodeling enzymes. One of the mechanisms is likely to happen through the previously referred role of mutant KRAS cancer cells in promoting the activation of tumor stromal fibroblasts, the recruitment of M1 macrophages and Th17 cells, which on their turn, impact on ECM composition and structure by secreting ECM components and MMPs (Fig. 1).

Besides this indirect link, a direct role of mutant KRAS on the modulation of ECM properties has also been described. In lung cancer, for example, epithelial cells expressing KRAS G12V mutation secreted higher levels of activated MMP-9, a significant player in ECM remodeling (Fig. 1; ref. 88). In pancreatic cancer mouse models, increased expression of MMP3 cooperates with KRAS activation to shape the stromal microenvironment, not only by stimulating immune cell influx but also as a primary proteolytic activator of MMP-9 (89). Also in pancreatic cancer cells, mutational activation of KRAS induced the expression of the eukaryotic translation initiation factor 5A (eIF5A) and consequent stimulation of ROCK1 and ROCK2 (90). Cell-based assays demonstrated that ROCK activation and signaling drives a gene expression program that results in ECM remodeling and collagen degradation by MMPs, thereby enabling invasive tumor growth through elimination of physical constraints (Fig. 1; ref. 91). Besides local induced ECM changes, conditional activation of KRAS G12D in the mouse urothelium triggered lung ECM defects, particularly abnormalities in some constituents of the basement membrane, laminin and nidogen, as a consequence of ECM degradation (92).

The described local and systemic KRAS-mediated effects on ECM are likely to impact on the motility, invasive, and metastatic capacity of tumor cells. As such, it illustrates another possibility to impair KRAS-mutant cancer cell dissemination, neutralizing tumor progression.

Concluding Remarks and Perspectives

The evidence linking KRAS signaling to tumor microenvironment modulation increases our understanding of the function of KRAS in cancer. Still, further studies are essential for a comprehensive understanding of the interactions between these cells and their microenvironment. For example, the specific literature focuses mainly on pancreatic and lung cancers, overlooking other types of cancers such as colorectal cancer, in which KRAS mutations are also frequent. A complete characterization of the paracrine effects of KRAS-mutant cancer cells in the tumor models with mutant KRAS would be valuable to obtain an overall view of these effects and to identify tumor specificities. This knowledge would be crucial to stratify and pinpoint the patients that may benefit from KRAS signaling–directed therapies or from its combination with stromal modulatory approaches. In addition, because metastatic outgrowth relies on the recruitment of noncancer cells, such as myeloid cells, endothelial cells, fibroblasts (93), and ECM remodeling (10), it would be relevant to address the role played by mutant KRAS cancer cells in the regulation of these stromal components in the target organ, and how this would impact on the establishment of the metastatic lesions. Also, because it is known that different KRAS mutations have distinct transforming potential and activate different transcriptional profiles (94), it would be relevant to understand whether they also induce different effects on the interaction between cancer cells and the microenvironment, and which signaling pathways are used. This would be relevant for the development of mutation-specific therapeutic approaches. In addition, and adopting the concept of “dynamic reciprocity” proposed by Bissel and colleagues to describe the mutual regulation of cancer cells and ECM (meaning that cancer cell communication with the microenvironment is not a unidirectional process; ref. 95), it would also be relevant to study the role of mutant KRAS on the integration of external signaling and on the subsequent cancer cell response. Ultimately, the identification of key molecules mediating this cross-talk will have a major impact on the design of new therapeutic strategies aiming to target KRAS-mutant cancer cells by abrogating their interactions with the microenvironment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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