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REVIEW

Molecular genetics and cellular events of K-Ras-driven tumorigenesis

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Cellular transformation and the accumulation of genomic instability are the two key events required for tumorigenesis. K-Ras (Kirsten-rat sarcoma viral oncogene homolog) is a prominent oncogene that has been proven to drive tumorigenesis. K-Ras also modulates numerous genetic regulatory mechanisms and forms a large tumorigenesis network. In this review, we track the genetic aspects of K-Ras signaling networks and assemble the sequence of cellular events that constitute the tumorigenesis process, such as regulation of K-Ras expression (which is influenced by miRNA, small nucleolar RNA and lncRNA), activation of K-Ras (mutations), generation of reactive oxygen species (ROS), induction of DNA damage and apoptosis, induction of DNA damage repair pathways and ROS detoxification systems, cellular transformation after apoptosis by the blebbishield emergency program and the accumulation of genomic/chromosomal instability that leads to tumorigenesis.

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INTRODUCTION

Cancer remains a difficult disease to cure and the most common cause of disease-associated mortality. An incomplete understanding of the tumorigenesis process and the generation of heterogeneity within tumors renders therapeutic measures ineffective against cancer. Accumulating evidences point to the fact that the clones that develop resistance to therapy are cancer stem cells[1] and often have genetic alterations in K-Ras.[2] One major reason for heterogeneity/clonal difference is genomic instability, which generates cancer cells with structural or numeric chromosomal alterations that result in alterations to critical genes such as K-Ras[3] that govern cell survival and immune evasion. In this review, we track the genetic aspects and sequence of cellular events that constitute the K-Ras-driven tumorigenesis process as a model for oncogene-driven tumorigenesis, with special focus on the role of cellular transformation and genomic instability and how RNA interference of K-Ras influences tumorigenesis.

K-RAS: GENETIC REGULATION OF EXPRESSION AND ACTIVATION

Ras oncogenes (*HRAS, KRAS* and *NRAS*) were discovered from viruses and were found to have transforming (sphere/focus-forming/anchorage-independent growth/clonogenic growth) properties.[4] *KRAS* encodes a 21 kDa protein product and is a powerful member of the Ras oncogene family. *KRAS* has two alternatively spliced mRNA variants, namely, KRAS4A and KRAS4B.[4] Human cells harbor the *KRAS* gene at chromosomal band 12p12.1.[5]

Once the *KRAS* gene is transcribed, the mRNA is either subjected to translation or RNA interference-mediated degradation (Figure 1 and Table 1). Let-7 micro-RNA (miR/miRNA) targets K-Ras mRNA for degradation through LCS (Let-7 complementary sites) within the K-Ras mRNA.[6,7] Interestingly, Chin *et al.*[8] used clinical specimens to identify that an LCS6 variant allele with a mutation in the miRNA-binding region was associated with K-Ras overexpression. H19 long non-coding RNA (lncRNA) blocks K-Ras mRNA degradation by antagonizing Let-7 miRNAs.[9] Moreover, miR-16 directly targets the 3′-UTR of KRAS mRNA to block K-Ras expression[10] (Figure 1). The extent to which miRNAs cross react with mRNAs of other ras oncogenes is not well understood. However, miRNA-18a was found to specifically target K-Ras mRNA without targeting transcripts of N-Ras or H-Ras[11] (Figure 1). Furthermore, miRNA-622,[12] miR-1/hsa-miR-1,[13] miR-217,[14] miR--143/145 (discussed below) and small nucleolar RNAs (discussed below) are also capable of targeting K-Ras expression (Figure 1).

Simply expressing K-Ras is not sufficient for tumorigenesis because when K-Ras was expressed under its own endogenous promoter with a conditional interferon inducible switch, it could only induce transformation and not acute myeloid leukemia.[15] Thus, the genetic aspects of *KRAS* promoter regulation is not discussed in detail here. Genomic instability in combination with cellular transformation was suggested to enable K-Ras-driven tumorigenesis.[15] This combination of cellular transformation and genomic instability also holds true for human papillomavirus-induced tumorigenesis.[16]

The expression of *KRAS* alone did not drive tumorigenesis because it did not account for the activation of K-Ras. When K-Ras is bound to guanosine diphosphate, it is in its inactive form, and when this guanosine diphosphate is replaced with guanosine triphosphate, K-Ras becomes activated.[4] Activated cell surface receptors (usually receptor tyrosine kinases/RTKs) activate K-Ras. RTKs and Ras signaling collaborate to develop specific sub-types of cancer and thus are important for cancer therapy.[17] Apart from

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protein-based activation, K-Ras also becomes constitutively activated by mutations in critical codons. High-frequency mutations (in clinical specimens), such as G12A, G12C, G12D, G12S, G12V, G13C and G13D, in addition to many other low-frequency mutations, are known to activate K-Ras[18–23] (Figure 1 and Table 1). These mutations interfere with guanosine triphosphate hydrolysis to make K-Ras constitutively active.[24,25] However, this concept was recently questioned because the binding of guanosine triphosphate to mutant K-Ras may vary depending on the type of mutation.[26] The K-Ras^{G13D} mutation directs ZNF304-DNMT1-mediated repression of tumor suppressor genes through promoter methylation.[27] K-Ras mutations are frequently observed in cancers of the pancreas (57%), large intestine (33%), biliary tract (31%), small intestine (20%), lung (17%), endometrium (14%), ovary (14%), prostate (8%), cervix (7%), stomach (6%), urinary tract (5%), liver (5%), haematopoietic cells (5%) and other organs ($<5\%$).[4] However, this could be an underestimation of the actual K-Ras activation status in cancer because these percentages only account for the mutant *KRAS* and not receptor-activated wild-type K-Ras. Mutations enable the oncogenic characteristics of both splice variants of *KRAS* (KRAS4A and KRAS4B).[28] Wild-type K-Ras activation modules are protein-based modifications and will not be discussed in detail here. For more details on this and on small molecule inhibition of K-Ras, please see the latest excellent review.[29]

Epidermal growth factor receptor (EGFR), one of the prominent candidates implicated in cellular transformation, is tightly linked to K-Ras-induced pancreatic tumorigenesis.[30] In addition, VEGFR2/KDR expression and PDGFRA expression were found to correlate

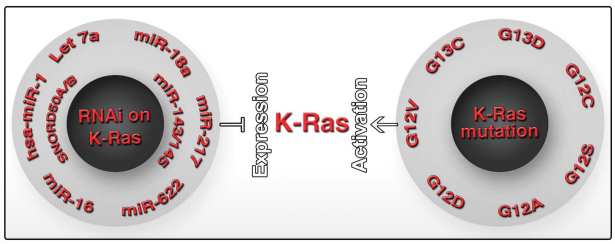

Figure 1. Key genetic factors regulating *KRAS* expression and K-Ras activation. Key miRNAs/snoRNAs targeting *KRAS* mRNA and inhibiting K-Ras expression are shown on the left, and the most frequent and prominent K-Ras mutations leading to constitutive K-Ras activation are shown on the right (see text for references).

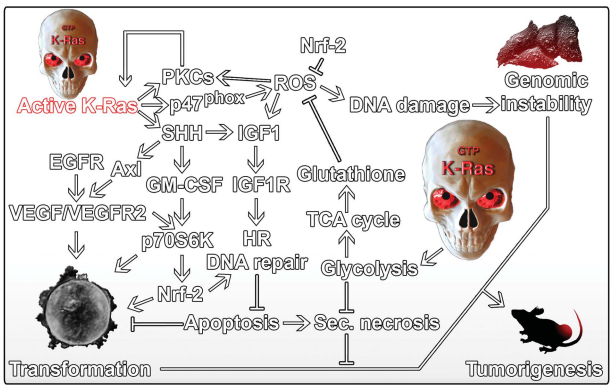

Figure 2. Cellular transformation and genomic instability are required for K-Ras-driven tumorigenesis. Key signaling pathways from activated K-Ras that lead to cellular transformation and genomic instability in various cancers. EGFR, epidermal growth factor receptor; GM-CSF, granulocyte monocyte-colony stimulating factor; HR, homologous recombination; IGF, insulin-like growth factor; PKC, protein kinase-C; ROS, reactive oxygen species; SHH, sonic hedgehog; TCA cycle, tri-carboxylic acid cycle; VEGF, vascular endothelial growth factor.

Table 1. Genetic and cellular events that regulate K-Ras-driven tumorigenesis

Major event	*Negative regulation*	*Positive regulation*
K-Ras expression	miRNAs (Let-7a, miR-18a, miR-217, miR-622, miR-16-1, hsa-miR-1, miR-143/145), miR-30a and snoRNAs (SNORD50A, SNORD50B).	lncR-H19-mediated inhibition of miRNA Let-7a.
K-Ras activation	DNA repair pathways that reverse K-Ras codon mutations.	Codon mutations (G12V, G12C, G12S, G12A, G12D, G13C and G13D), RTKs, smoking and ROS
K-Ras-regulated non-coding RNAs	Potential miRNA target (miR-1298).	miRNAs (miR-21, and miR-214), and lncR-ANRIL.
ROS generation	Smac mimetics + TNF-α combination, BI-D1870, NAC, glutathione, NQO1 and Nrf-2.	K-Ras interaction with p47^{phox}, PKCs (PKC-ζ), and Ser-181-phosphorylation of K-Ras.
Apoptosis induction	Antioxidant system (Nrf-2, SLC7A5, HMOX-1, NQO1), IRES translation of c-IAPs (c-IAP1/2, XIAP) and induction of DNA repair pathways.	ROS-induced DNA damage
Secondary necrosis	BAD-Ser-112 phosphorylation by p70S6K and Pim-1 to boost glycolysis to generate ATP.	Lack of ATP after glycolytic shut down.
Blebbishield formation	Dynasore (Dynamin-dependent endocytosis inhibitor), and *N*-ethylmaleimide (NEM).	Dynamin-dependent endocytosis, serpentine filopodia formation and membrane fusion.
Blebbishield-mediated transformation	Neutralizing antibodies to VEGF-A and VEGFR2.	VEGF-A, VEGFR2, cell fusion, lactic acid, IRES translation and p70S6K.
Genomic instability	Mitochondrial apoptosis through wild-type p53, p21, Bax/Bak, necrosis and senescence through pRb.	Mutant p53 or loss of p53, cell fusion, mitotic defects (cytokinesis failure), DNA repair defects induced by K-Ras, blebbishield emergency program and chromosomal instability.
Tumorigenesis	miRNAs (miR-216, miR-1298, miR-143/145, miR-16, Let-7a, miR-214, hsa-miR-1, miRNA-622, miR-217, miR-30a), induction of proper immunogenic apoptosis (wild-type p53, p21, cleaved Bax/Bak) and senescence (pRb) and snoRNAs (SNORD50A, SNORD50B).	K-Ras expression and activation, miRNAs (miR-21, miR-26a), lncRNA ANRIL, FAK, ERK-1/2, glycolytic switch, cellular transformation, genomic instability, evasion of apoptosis and immunity, and overriding cell cycle checkpoints.
Cancer therapy	Blebbishield emergency program (inhibits cell death), glycolysis (inhibits secondary necrosis after apoptosis) and lack of functional wild-type p53.	K-Ras inhibitors, miRNA therapeutics, glycolysis inhibitors with apoptosis inducers, immunotherapeutics and other preclinical therapeutics.

with K-Ras codon 12 and 13 mutations in colorectal cancer.[31] K-Ras^{G12D} mutant cells regulate granulocyte monocyte-colony stimulating factor and granulocyte-colony stimulating factor secretion through sonic hedgehog-dependent recruitment of tumor stroma.[32] All these factors govern cellular transformation and genomic instability to cause tumorigenesis (Figure 2). Sonic hedgehog induces IGF1 and GAS6 to activate IGF1R and AXL/TYRO3, respectively, leading to downstream AKT (pT308/pS473), MEK and IRS-1 activation.[32] Often, K-Ras mutations mimic the activation of RTKs and thus cause resistance to most RTK inhibitors because RTKs are upstream of K-Ras activation. For example, K-Ras mutation contributes resistance to IGF1R tyrosine kinase inhibitors.[33] On the other hand, the inhibition of AXL suppresses DNA damage responses and sensitizes cancer cells to PARP inhibitors.[34] Thus, K-Ras either directly or indirectly drives the resistance of cancer cells to therapeutic agents that target RTKs (Figure 2).

K-RAS: INDUCTION OF ROS, APOPTOSIS AND ROS DETOXIFICATION IN CELLULAR TRANSFORMATION

Once activated, K-Ras has a plethora of functions; however, the induction of reactive oxygen species (ROS) is the key step toward cellular transformation, genomic instability and tumorigenesis. K-Ras orchestrates ROS production by promoting the localization of NOX1 component p47^{phox} to the plasma membrane and facilitates its interaction with protein kinase-C isoforms (PKC) to mediate cellular transformation[35] (Figures 2 and 3 and Table 1). In addition, K-Ras activation is linked to an altruistic positive feedback loop between PKC-ζ, p47phox, and ROS wherein K-Ras facilitates p47^{phox} to generate ROS, and ROS offers sustained activation status for PKC-ζ because PKC-ζ and p47^{phox} are interacting partners[36] (Figures 2 and 3). PKCs in turn phosphorylate both K-Ras isoforms (KRAS4A and KRAS4B) at Ser-181 to activate K-Ras.[37,38] ROS production is, however, deleterious to cells as it can damage DNA through oxidative stress and induce apoptosis. Paradoxically, K-Ras can induce apoptosis, as well as promote tumorigenesis.[39] Although these functions are contradictory in nature, ROS-induced apoptosis[36,40] and K-Ras/VEGF/p19-VHL-directed survival functions (ROS neutralization by *IGFBP3, SLC7A5* and *SLC3A2*[41]) are utilized in sequence to complete cellular transformation by the blebbishield emergency program.[42–44] Pim kinase isoforms are instrumental in protecting mitochondria from damage because loss of Pim kinase isoforms correlates with damage to mitochondria and the inhibition of cellular transformation.[40] Inhibition of ROS by targeting PKC-ζ and p47^{phox} abrogates cellular transformation.[36] Thus, persistent altruistic feedback from ROS is needed to keep PKC-ζ active to regulate the expression of survival signals such as vascular endothelial growth factor (VEGF) and c-IAP2 through internal ribosome entry site (IRES) translation (Figure 3).

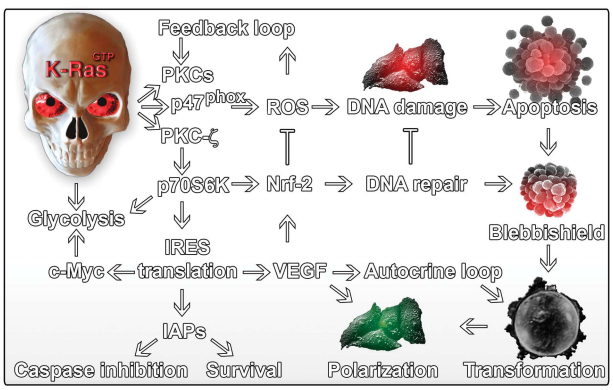

Figure 3. Cellular transformation after apoptosis by the blebbishield emergency program. Key signaling pathways from activated K-Ras that lead to cellular transformation by promoting glycolysis, inhibiting apoptosis through IAPs and ROS detoxification. IAPs, inhibitor of apoptotic proteins (XIAP, cIAP-1/2); IRES, internal ribosome entry site; PKC, protein kinase-C; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor.

Persistent ROS generation can damage DNA and can activate p53 to promote full-blown apoptosis.[45,46] To prevent p53 activation, K-Ras must either control the level of ROS generation or neutralize ROS. ROS detoxification is one way by which K-Ras and K-Ras^{G12D} overcome the deleterious effects of ROS. K-Ras regulates the expression of a panel of ROS detoxification mediators such as Nrf-2,[47] NQO1,[48] and HMOX-1 to promote K-Ras-driven tumorigenesis[49] (Table 1). Expression of the chief ROS detoxification mediator Nrf-2 in response to ROS is under the control of protein translation through p70S6K,[50] which in turn is a downstream target of PKC-ζ[51] (Figure 3). Thus, constitutive K-Ras activation leads to the constitutive activation of an altruistic feedback loop of ROS generation and sustained PKC activation, in which PKC-ζ drives the activation of p70S6K and regulates the expression of Nrf-2 to neutralize the toxic effects of ROS (Figure 3).

K-RAS AND BLEBBISHIELD-MEDIATED CELLULAR TRANSFORMATION AFTER APOPTOSIS

Soft-agar transformation assay has been the method of choice for decades to test the transforming ability of individual oncogenes. K-Ras knock-in was found to transform non-tumorigenic cells.[52] Interestingly, soft-agar transformation is a long assay and takes days to weeks to reach an endpoint. Hence, the short-term events during cellular transformation were obscured for a long time. In the quest to understand cancer stem cells between low and high tumorigenic versions of bladder cancer cells, it was found that sphere formation (transformation) was preceded by apoptosis and a subsequent blebbishield emergency program, which rescues apoptotic cells and redirects them toward K-Ras-mediated cellular transformation[36,42,43,53] (Figure 3).

Smac mimetics in combination with death ligands such as tumor necrosis factor-α or tumor necrosis factor-related apoptosis inducing ligand were found to effectively abrogate K-Ras activation, leading to massive cell death in cancer cells.[44] Although FasL in combination with a Smac mimetic could induce apoptosis in cancer cells, a subset of apoptotic cells managed to survive after apoptosis and underwent cellular transformation (sphere formation) using the blebbishield emergency program.[44] The blebbishield emergency program constructs blebbishields from apoptotic bodies using dynamin-dependent endocytosis-driven serpentine filopodia, and the blebbishields in turn fuse to each other to undergo cellular transformation.[36,42,43,53–55] The transformation phase of the blebbishield emergency program is driven by VEGF-activated VEGFR2 to activate K-Ras[43,44] (Table 1). K-Ras signaling activates p70S6K (through ROS, PKC-ζ axis) to enhance IRES translation of VEGF to activate the VEGF autocrine loop[44] (Figure 3). The blebbishields sustain VEGF secretion by maintaining p70S6K activation and thus continue IRES translation even during apoptosis.[44,56] IRES translation also provides survival signals in the form of inhibitor of apoptotic proteins (IAPs)[44] (Figure 3 and Table 1). DNA damage response is very important in the context of the blebbishield emergency program because DNA damage is a hallmark of apoptosis. The Nrf-2-ROS detoxification system has a direct role in activating DNA repair through homologous recombination[57] (Figure 3).

K-Ras was found to be in an oligomerized state in parallel to BAD, Bax, Bak and p27 to boost glycolysis, so that the apoptotic cells were not subjected to secondary necrosis during the blebbishield emergency program.[44] This could be one of the main reasons why cancer cells prefer glycolysis. Active K-Ras, p70S6K and Pim kinase-1 isoforms were found to have pivotal

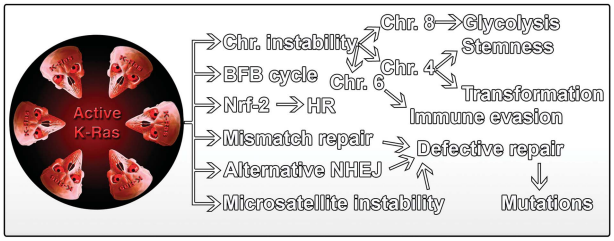

Figure 4. K-Ras-driven genomic instability in tumorigenesis. K-Ras hijacks DNA repair pathways either to make defective repair or to promote survival after apoptotic DNA damage. Chr., chromosome; HR, homologous recombination; NHEJ, non-homologous end joining.

roles in blocking BAD-mediated apoptosis by maintaining the phosphorylation of BAD at its Serine-112 gatekeeper site.[40,56] Compromising this phosphorylation using Smac mimetics plus tumor necrosis factor-α/tumor necrosis factor-related apoptosis inducing ligand leads to the abrogation of the transformation phase of blebbishield emergency program by promoting the depolarization of mitochondria and the induction of complete apoptosis[40,58,59] (Table 1). Similarly, interfering with Ser-181 phosphorylation of K-Ras4A and K-Ras4B isoforms blocks tumorigenesis and cellular transformation (foci formation), respectively.[37,38]

The blebbishield emergency program explains why K-Ras and BAD facilitate both apoptosis and cell survival,[39,60] because apoptosis induction and subsequent survival are necessary for cellular transformation.[42,61,62] In support of this notion, PARP inhibitors and the caspase-3 inhibitor z-DEVD-fmk were found to abrogate cellular transformation.[44,63] Furthermore, soft-agar transformation and blebbishield emergency program-mediated cellular transformation are driven by an identical mechanism because a small molecule, CF3-DODA-Me, abrogates cellular transformation in soft-agar assays, as well as blebbishield-mediated sphere formation assay, by targeting drivers of the blebbishield emergency program.[64]

K-RAS: GENOMIC INSTABILITY, THE NEXT CRUCIAL STEP TOWARDS TUMORIGENESIS

Cellular transformation that is induced by K-Ras is not enough to drive tumorigenesis. For efficient tumorigenesis, cells need to accumulate mutations or genomic instability.[15] This is, in fact, demonstrated in the case of human papillomavirus-induced tumorigenesis in which transformation and genomic instability together drive tumorigenesis.[16] Thus, the generation of genomic instability is the next key step in K-Ras-driven tumorigenesis. K-Ras activation causes early tumorigenesis in a mouse model of lung cancer,[65] suggesting the capability of K-Ras to induce both transformation and genomic instability. Genomic instability can occur within a specific location of the genome (for example, microsatellite instability) or at the chromosomal level as structural and/or numeric alterations (such as ploidy/aneuploidy, breakage-fusion-breakage/breakage-fusion-bridge cycle between chromosomes, or the translocation of chromosomal arms, resulting in fusion genes) (Figure 4). Even though cells have DNA repair mechanisms, defective DNA repair can lead to genomic instability.

K-RAS: DNA REPAIR DEFECTS AND GENOMIC INSTABILITY

Mismatch repair (MMR) is a small-scale DNA repair mechanism in cells. Defective MMR can lead to mutational hotspots/microsatellite instability and could be the cause of K-Ras activating mutations (Figure 4). EGFR is known to phosphorylate PCNA to inhibit MMR;[66] therefore, it can increase mutation burden. In a pancreatic cancer mouse model, K-Ras upregulated EGFR expression, and genetic interference of EGFR eliminated K-Ras induced tumorigenesis.[30] Thus, K-Ras is associated with microsatellite instability in cancer,[67,68] which might require other factors such as PCNA inhibition by EGFR to lead to defective MMR in K-Ras-driven tumorigenesis.

DNA damage that is induced during apoptosis must be repaired by cells to override cell death and to undergo cellular transformation. To ensure protection from DNA damage, K-Ras-activated cells utilize various tactics, including the Nrf-2-dependent ROS detoxification system, which not only neutralizes ROS but also activates homologous recombination-mediated DNA repair[57] (Figure 3). Telomere shortening-induced exposure of naked DNA ends at telomeric regions of chromosomes results in chromosome–chromosome fusion by the non-homologous end joining (NHEJ) mode of DNA repair to form breakage-fusion-bridge cycle (BFB cycle) type chromosomes.[69,70] The classical NHEJ pathway contributes to genomic instability during mitosis if X-ray repair cross-complementing protein 4 (XRCC4) is not phosphorylated at the mitotic phase (M-phase). However, K-Ras targets an alternative XRCC4-independent NHEJ error-prone pathway that includes DNA ligase-3α, Poly (ADP-ribose) polymerase 1 and XRCC1 as components.[71] Mutant K-Ras is known to upregulate the components of this error-prone alternative NHEJ pathway (DNA ligase-3α, Poly (ADP-ribose) polymerase 1 and XRCC1), and cells depend on this alternative NHEJ pathway during genotoxic stress.[71] Thus, the MMR, homologous recombination and NHEJ modes of DNA repair can all be misused by K-Ras to generate genomic instability (Figure 4 and Table 1).

K-RAS: CHROMOSOMAL INSTABILITY IN METABOLIC REPROGRAMMING, SURVIVAL AND STEMNESS

Chromosomal instability is a large-scale genomic alteration with numerical or structural alterations in chromosomes. Deleting p53 in non-tumorigenic epithelial cells resulted in the generation of transformed phenotype and chromosomal instability.[72] Rodrigues *et al.*[73] also found that chromosomal instability is associated with cellular transformation. Thus, a transformation event can also generate chromosomal instability if p53 is compromised. *KRAS* mutation coupled to p53 loss can lead to tumorigenesis.[74] Recently, blebbishield emergency program is found to drive severe chromosomal instability by suppressing the expression of p53 during the transformation phase.[61] In addition, EGFR, a K-Ras target, induces the expression of miR-26a in non-small cell lung cancer,[75] and miR-26a promotes chromosomal instability and tumorigenesis in breast cancer cells.[76]

The accumulation of chromosome-8 has been predominantly correlated with genomic instability in multiple cancer types[77] (Figure 4). Chromosome-8 harbors the Myc gene,[78] one of the important targets of K-Ras (through the regulation of IRES translation).[44] K-Ras and Myc are regulators of glycolysis to avert secondary necrosis of apoptotic cancer stem cells during blebbishield-mediated transformation[44] (Figure 3). Thus, the control of K-Ras over metabolic reprogramming[79] has a potential genetic basis, the chromosome-8 accumulation. Alternatively, mutant K-Ras^{G12D} copy number influences glucose metabolism and the TCA cycle to generate glutathione for ROS detoxification.[80] Furthermore, the glycolysis metabolite lactic acid (lactate) offers strong survival advantages to cancer stem cells that undergo cellular transformation using the blebbishield emergency program by reducing the pH and potentially increasing the bioavailability of VEGF from the microenvironment.[42] Furthermore, targeting lactate dehydrogenase-A (an enzyme that converts pyruvate to lactate) has been shown to impede cellular transformation[81] and tumorigenesis.[82] Thus, the blebbishield emergency program positively selects cells that are capable of

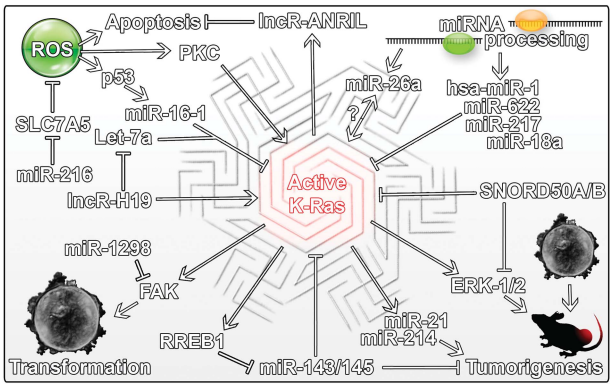

Figure 5. RNA interference that impedes or promotes K-Ras-driven tumorigenesis. Schematic showing RNA interference that inhibits or promotes K-Ras-driven tumorigenesis by targeting cellular transformation, genomic instability or K-Ras. FAK, focal adhesion kinase; lncR, long non-coding RNA; miR, micro-RNA; ROS, reactive oxygen species.

enhanced glycolysis, lactate production and survival after apoptosis.

In addition, K-Ras has been linked to the accumulation of chromosomes-4, 10 and 12 in colorectal carcinomas.[83] Among these, chromosome-4 harbors VEGFR2/KDR, c-Kit and PDGFRA in the same cytogenetic band (4q12) as a cluster where VEGFR2/KDR expression and PDGFRA expression were found to correlate with K-Ras codon 12 and 13 mutations in colorectal cancer.[31] This is very important because VEGF/VEGFR2 signaling drives the blebbishield emergency program for cellular transformation[43,44] (Figures 2 and 3). Chromosome-4 accumulation could also provide advantages in stemness (owing to c-Kit[42]) and survival (owing to IRES translation of anti-apoptotic factors such as c-IAP2, XIAP, Tau through the VEGFR2/p70S6K axis[44]) (Figure 3 and Table 1).

Interestingly, the selective loss of chromosome-6 was found to be a cause of K-Ras^{G12D} mutant-mediated immune evasion.[2] Thus, chromosome loss can also contribute to K-Ras-driven tumorigenesis and immune evasion.

MICRO-RNA, lncRNA, snoRNA AND REGULATION OF K-RAS-DRIVEN TUMORIGENESIS

In general, impaired micro-RNA processing is linked to tumorigenesis.[84] Interestingly, p53 acts as a switch regulating miRNA processing.[85] ROS is known to induce the expression and activation of p53 to induce apoptosis,[46] and hence, miRNA targeting of ROS detoxification mechanisms should help to induce p53 expression or act against K-Ras. In this context, SLC7A5, a ROS detoxification mediator, is a target for miR-216 (Figure 5), and miR-216 targets multiple mediators of K-Ras-driven tumorigenesis to block K-Ras-dependent survival[86] (Table 1). Similarly, miRNAs that act as indirect targets also impede or enhance K-Ras-driven tumorigenesis. For example, miR-1298 inhibits K-Ras-driven tumor growth by targeting focal adhesion kinase[87] (Figure 5). In NSCLC, miR-21 enhances K-Ras-driven tumorigenesis by targeting negative regulators of the Ras/MAPK pathway.[88]

In addition, miRNAs that directly target K-Ras signaling also impede or enhance K-Ras-driven tumorigenesis. For example, miR-143/145 blocks K-Ras-mediated tumorigenesis, whereas K-Ras activation suppresses the expression of miR-143/145 through Ras-responsive element-binding protein[89] (Figure 5). K-Ras expression and tumorigenesis are inhibited by miR-16, which directly targets the 3′-UTR of *KRAS* mRNA.[10] Let-7a targets K-Ras to inhibit the malignant property of glioblastoma cells.[7] Let-7 miRNA has been implicated in inflammation associated cellular transformation[90] and targeting K-Ras[6] (Figure 5). H19 long non-coding RNA antagonizes Let-7 miRNA[9] and increases K-Ras expression by reversing Let-7-mediated miRNA interference (Figure 5). In this context, H19 long non-coding RNA regulates cellular transformation and potentiates breast and lung tumorigenesis.[91] An LCS6 variant allele associated with K-Ras overexpression owing to a mutation in the miRNA-binding site increased the risk of lung cancer among smokers.[8] Of note, smoking induces spontaneous mutations within the *KRAS* gene.[92] In a spontaneous lung cancer mouse model driven by *K-Ras^{G12D}* and *p53$^{-/-}$* genotype, miRNA-214 was found to be overexpressed. Impeding miR-214 blocked tumor growth to demonstrate the tumor-promoting role of miR-214 in a K-Ras mutant system[93] (Figure 5). In breast cancer, miR-1 (hsa-miR-1) targets K-Ras to impede tumor growth and metastasis, thereby acting as a tumor suppressor.[13] In addition, miRNA-622 targets K-Ras to inhibit transformation *in vitro* and tumorigenesis *in vivo*[12] (Figure 5). Similarly, miR-217 targets K-Ras to inhibit cellular transformation and tumor growth in pancreatic ductal adenocarcinoma of mice[14] (Figure 5). In glioblastoma multiforme, K-Ras expression was found to be necessary to maintain tumors *in vivo,* and loss of K-Ras led to apoptotic clearance of these cells.[94] K-Ras was found to influence the expression of the long non-coding RNA ANRIL,[95] which promotes lung cancer by inhibiting apoptosis[96] (Figure 5).

K-Ras also exerts its oncogenic effects through ERK-1/2[97] and is negatively regulated by the small nucleolar RNA SNORD50A, and SNORD50B-mediated direct targeting of K-Ras and CRISPR/Cas9-mediated deletion of these small nucleolar RNAs enhance tumorigenesis.[98]

Taken together, these studies reiterate and confirm the fact that cellular transformation and genomic instability are two prominent features that are essential for K-Ras-driven tumorigenesis and highlight the dominance of genetic factors in K-Ras-driven tumorigenesis.

SUMMARY AND FUTURE PERSPECTIVES

K-Ras induces apoptosis through oxidative stress and then detoxifies ROS to induce DNA damage repair to regulate cellular transformation through the blebbishield emergency program and induces genomic instability to drive tumorigenesis. K-Ras could possibly mediate therapy resistance and relapse through the blebbishield emergency program because chemoresistant cancer clones were found to have a link to K-Ras.[5] K-Ras in general is known to suppress the apoptotic program of death receptors and to redirect cells toward metastasis,[99] which explains the importance of the blebbishield emergency program.[44] In this context, inhibition of CDK4/6 gains importance as it could increase sensitivity to paclitaxel in K-Ras mutant cells[100] or induce senescence and impede tumor growth with autophagy inhibitors.[101]

A new avenue of miRNA therapeutics targeting K-Ras (based on tumor suppressor miRNAs Let-7a, miR-143, miR-145 and miR-200 family) is emerging at the preclinical level, highlighting the importance of K-Ras miRNA regulatory networks in cancer therapy.[102] Although the expression of miR-26a in lung cancer suggests a link to K-Ras activation, the nature of this regulation remains unexplored (Figure 5). The introduction of miRNA-zippers, which lock two miRNAs together to inactivate both miRNAs[103] and iExosomes[104] (exosomes with siRNA or shRNA targeting KRAS^{G12D} mRNA), provides more choices for miRNA-based therapeutics. Thus, evaluating K-Ras in cancer relapse and expanding miRNA/RNAi therapeutics against K-Ras-driven cancers are attractive and emerging avenues of research. Despite smoking being linked to mutational activation of K-Ras and driving tumorigenesis in the context of p53 mutations,[105] an evaluation of oncogenic viruses in the induction of K-Ras mutations is much needed. This review will also serve as a model to explore the mechanism of other

oncogenes in various cancers for better understanding and better therapeutic development in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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