A-Raf: A serine/threonine protein kinase with important biological functions (Review)

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Abstract. A-Raf is a serine/threonine protein kinase that belongs to the RAF kinase family. A-Raf serves key roles in various physiological and pathological processes, including cell cycle regulation, apoptosis, material transport and metabolism, embryonic development, bone mass maintenance and tumor progression. As research has advanced, the functional mechanisms of the A-Raf gene have attracted increasing attention. To systematically summarize its biological functions, the present review focuses on the effects of A-Raf on the cell cycle, apoptosis and cell proliferation, its influence on cellular material transport and metabolic processes, its role in organismal development and bone mass maintenance, its impact on tumor progression, the effects of genetic variations on A-Raf function and the regulation of A-Raf by upstream factors. The present review therefore aimed to provide a novel perspective for the comprehensive understanding of the function of A-Raf.

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Abbreviations: SH2, Src homology 2; DKO, double knockout; c-FOS, Fos proto-oncogene; TH1, trihydrophobin 1; MST2, mammalian STE20-like kinase 2; hnRNPH, heterogeneous nuclear ribonucleoprotein H; M2-PK, pyruvate kinase M2; HCC, hepatocellular carcinoma; FA2H, fatty acid 2-hydroxylase; GBC, gallbladder cancer; iCCA, intrahepatic cholangiocarcinoma; EIF5A1, eukaryotic translation initiation factor 5A-1; CK2β, casein kinase 2 β subunit

Key words: A-Raf, biological functions, cellular metabolism, genetic variation, regulatory mechanism

Contents

1. Introduction

A-Raf is a serine/threonine protein kinase encoded by the RAF family. Along with BRAF and CRAF (RAF1), it is one of the three major members of this family and serves a key role in cell cycle and apoptosis regulation, substance transport and metabolism, embryonic development, bone maintenance and tumor progression (1-5). The A-Raf gene is located on band 5 of the long arm of chromosome Xand its protein product is structurally highly homologous to BRAF and CRAF (6,7). Tissue expression profiles have shown that A-Raf exhibits marked tissue specificity: A-Raf is most abundant in the epididymis, followed by the intestine (8). A-Raf interacts with human translocase of the outer membrane (hTOM) on the cytoplasmic face of the mitochondrial outer membrane and with human translocase of the inner mitochondrial membrane (hTIM) on the inner mitochondrial

membrane. hTOM and hTIM act synergistically to mediate A-Raf transport into mitochondria, where it is imported and anchored (9). In mouse models, A-Raf is located in the proximal region of the mouse X chromosome and exhibits distinct segmental expression in the epididymis: A-Raf expression was found to be higher in the proximal segment of the epididymis compared with the distal segment, with the lowest level having been detected in the initial segment; *in situ* hybridization results have also further demonstrated this gradient distribution pattern (10-12).

The promoter region of the human A-Raf gene contains three functional glucocorticoid response elements (GREs), namely GRE-1, GRE-2 and GRE-3, which are located at -17, -34 and -168 bp, respectively, from the transcription start site (13). The Src homology 2 (SH2) domain of the 85 kDa regulatory subunit of PI3K can bind directly to A-Raf. This interaction is independent of phosphorylation. The p85C-SH2 domain recognizes two distinct binding sites on A-Raf, with one overlapping with the classical phosphotyrosine-dependent site and the other being a phosphorylation-independent site (14). In addition, amino acids 248-267 of A-Raf form a novel regulatory sequence referred to as the isoform-specific hinge segment (IH-segment), which contains seven putative phosphorylation sites. Among these, Ser257, Ser262 and Ser264 synergistically enhance A-Raf activity, suggesting that the IH-segment serves an important role in the precise regulation of A-Raf function (15).

Based on research advances regarding A-Raf, the present review summarizes its effects on the cell cycle, apoptosis and cell proliferation, its roles in cellular transport and metabolic processes, embryonic development and bone homeostasis, its impact on tumor progression, the functional consequences of genetic variants and the influence of upstream regulatory factors. The present review therefore aimed to provide a novel perspective for the comprehensive understanding of A-Raf functions.

2. A-Raf affects the cell cycle, apoptosis and cell proliferation

With regard to cell cycle regulation, both A-Raf-Raf-1 double knockout (DKO) and trihydrophobin1 (TH1) can inhibit the cell cycle by modulating proliferation-associated proteins. DKO mouse fibroblasts have exhibited delayed entry into S phase and reduced proliferative activity, which is associated with decreased transient phosphorylation of MEK and ERK, as well as reduced expression levels of Fos proto-oncogene (c-Fos) and cyclin D1 (16). This suggests that A-Raf and Raf-1 jointly maintain normal cell cycle progression. Their functions are complementary, such that the absence of one can be compensated by the other, whereas simultaneous loss leads to severe impairments in proliferation and development (16). TH1 is an endogenous negative regulator of A-Raf that specifically binds to A-Raf and directly inhibits its kinase activity, thereby arresting cells in the G_0/G_1 phase by blocking the MAPK pathway and downregulating c-Fos and cyclin D1 expression (3,17).

A-Raf is a key signaling hub that regulates apoptosis (18). The subcellular localization of A-Raf depends on the scaffolding protein kinase suppressor of Ras 2 (KSR2). Elevated KSR2 expression promotes A-Raf translocation to mitochondria, where it binds to and inhibits the pro-apoptotic protein mammalian STE20-like kinase 2 (MST2), thereby blocking apoptosis (3,18). Heterogeneous nuclear ribonucleoprotein H (hnRNPH), acting as a transcriptional regulator, upregulates A-Raf transcription and expression (19). A-Raf exerts an anti-apoptotic effect by binding to and inactivating MST2 (19). *Toxoplasma gondii* infection, one of the prerequisites for the inhibitory expression of A-Raf in hosts, upregulates host microRNA-185 expression, which specifically suppresses A-Raf expression and markedly enhances the apoptosis of host cells (20).

A-Raf further serves a central role in the regulation of cell proliferation. A-Raf acts in concert with CRAF to mediate serum-induced proliferation in vascular smooth muscle cells (21). In an ovalbumin-induced asthma mouse model, baicalin suppressed the RAS signaling pathway by downregulating A-Raf expression, thereby inhibiting airway smooth muscle cell proliferation (22).

3. A-Raf affects cellular material transport and metabolic processes

A-Raf influences cellular transport processes through upstream regulation. The small G protein ARF GTPase 6 (ARF6) governs non-clathrin-mediated endocytosis and vesicular trafficking. As a key downstream effector kinase of ARF6, A-Raf specifically regulates the subsequent transport of endocytic vesicles, endosomal maturation and directed transport to the pericentriolar region (23).

A-Raf is also involved in the regulation of carbohydrate and lipid metabolism. A-Raf specifically binds to pyruvate kinase M2 (M2-PK) and regulates it in a non-kinase-dependent manner, inducing a shift from a highly active tetramer to a less active dimer, thereby inhibiting glycolysis and promoting the Warburg effect in tumor cells (24,25). In patients with hepatocellular carcinoma (HCC), the expression of A-Raf and fatty acid 2-hydroxylase (FA2H) is upregulated. On the one hand, A-Raf signaling activates the MAP2K1/ERK pathway to promote the proliferation of HCC cells. Meanwhile, the MAP2K1/ERK pathway upregulates FA2H expression by enhancing the expression of the activator protein-1 (AP-1) family. Upregulated FA2H promotes the production of 2-hydroxy fatty acid (hFA)-sphingolipids, which further increases A-Raf expression. This positive feedback loop accelerates HCC cell proliferation and induces increased expression of hFA-sphingolipids ultimately promoting the progression of HCC (Fig. 1) (26).

4. A-Raf serves important roles in organismal development and bone mass maintenance

A-Raf serves a role in regulating the development of the nervous system, the gastrointestinal tract and bones. A-Raf-knockout mice have been shown to reach perinatal mortality and exhibit severe defects in nervous system and gastrointestinal tract development. These defects cannot be compensated for by other Raf family members (B-Raf and C-Raf). This suggests that although A-Raf deficiency does not affect embryonic development, A-Raf serves an irreplaceable role in postnatal survival, neural development and

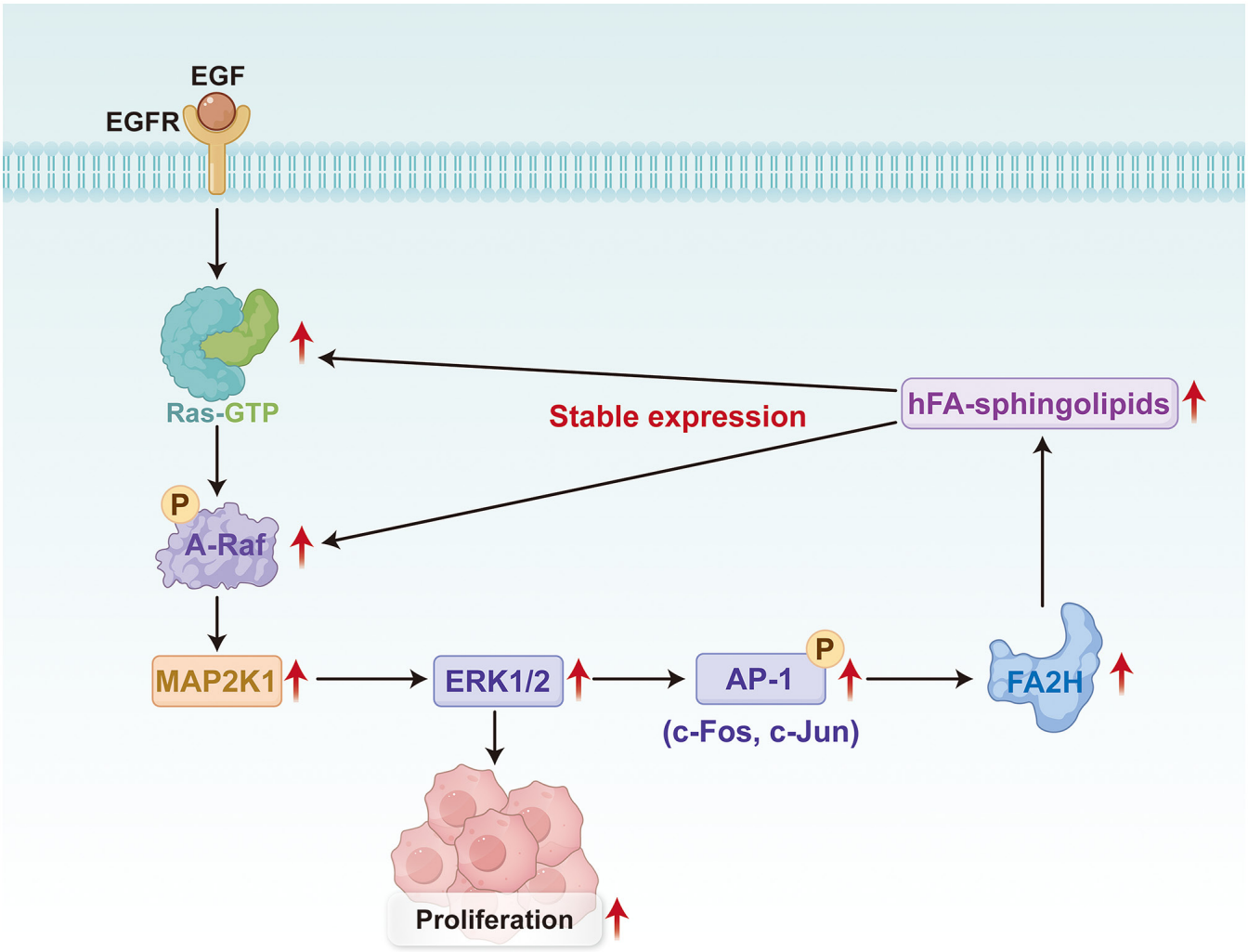

Figure 1. Co-upregulation of A-Raf and FA2H promotes cell proliferation and disrupts lipid metabolism in hepatocellular carcinoma cells. Binding of EGF to EGFR induces the conformational activation of Ras, converting it into its GTP-bound active form. Active Ras-GTP subsequently promotes the phosphorylation and activation of A-Raf. Activated A-Raf phosphorylates MAP2K1, which in turn phosphorylates and activates ERK1/2, thereby driving cell proliferation. ERK1/2 also mediates the phosphorylation of AP-1, which subsequently upregulates FA2H. FA2H catalyzes the biosynthesis of hFA-sphingolipids. These hFA-sphingolipids stabilize membrane signaling complexes comprising EGFR, Ras and A-Raf, thereby further amplifying MAP2K1 activation and downstream signaling cascades. FA2H, fatty acid 2-hydroxylase; hFA, 2-hydroxy fatty acid; AP-1, activator protein-1; P, phosphorylated; c-FOS, Fos proto-oncogene.

gastrointestinal homeostasis maintenance (5,27). A-Raf binds to SMAD2 and catalyzes the phosphorylation of specific sites within the SMAD2 linker region, thereby inhibiting SMAD2 nuclear translocation, attenuating its transcriptional activity, suppressing the expression of Nodal downstream target genes and causing endodermal defects in the embryo (28). A-Raf regulates the process of osteogenesis by modulating the expression of osteogenesis-associated genes (such as Runx2 and Sp7), thereby maintaining differentiation homeostasis and contributing to bone mass maintenance (29).

5. A-Raf affects tumor progression

A-Raf inhibits the progression of certain types of lung cancer. Particularly in lung cancer types harboring wild-type or single-mutation KRAS, elevated A-Raf expression suppresses erb-b2 receptor tyrosine kinase 3 (ERBB3) transcription and protein expression, thereby inhibiting lung cancer cell invasion, migration and distant metastasis (19).

A-Raf acts as an oncogene in HCC, pancreatic cancer, gallbladder cancer (GBC) and breast cancer (30-34). Heterogeneous nuclear ribonucleoprotein A2 (hnRNPA2) is highly expressed in HCC and regulates A-Raf alternative splicing by binding to A-Raf pre-mRNA. hnRNPA2 suppresses the generation of the dominant-negative short A-Raf isoform, upregulates full-length A-Raf expression and enhances MAPK pathway activity, thereby exerting oncogenic effects (30). In addition, 5'-tRNA-derived stress-induced RNA-glutamine downregulates A-Raf protein expression during HCC progression, thereby inhibiting proliferation and metastasis; further suggesting that A-Raf serves an oncogenic role in HCC (31). The long non-coding RNA linc01232 is highly expressed in pancreatic cancer and positively regulates A-Raf alternative splicing, resulting in increased production of full-length functional A-Raf; thus linc01232 constitutes an indispensable core oncogenic target in pancreatic cancer progression and metastasis (32). A-Raf expression is aberrantly upregulated in GBC tissues and cell lines. Following small interfering

RNA-mediated A-Raf silencing, GBC cell proliferation, clonogenicity, migration and invasion have been shown to be suppressed, whereas apoptosis is enhanced. These findings indicate that A-Raf functions as an oncogene to promote GBC progression (33). A-Raf and RAS form protein aggregates on the cell membrane, which shield RAS from inactivation by the tumor suppressor neurofibromin 1 (NF1) and continuously drive breast cancer cell proliferation. Furthermore, increased A-Raf-RAS aggregate formation confers endocrine therapy resistance in hormone-sensitive breast cancer (34).

6. Effects of genetic variations on A-Raf function

In the cytoplasm, Raf exists as a monomer. Phosphorylation of the serine residue in the conserved region 2domain enables binding to 14-3-3 dimers, thereby maintaining a Raf autoinhibited state. Mutations in this region disrupt 14-3-3 binding, resulting in constitutive Raf activation (35). The gain-of-function mutation A-Raf-S214P is a causative factor in central conductive lymphatic anomaly. Mutant A-Raf markedly upregulates ERK1/2 activity, enhances lymphangiogenesis, disrupts the linkage between the actin cytoskeleton and vascular endothelial cadherin, leading to abnormal lymphatic vessel structure and leakage (36). A-Raf-S214C is a novel driver mutation in lung adenocarcinoma; however, only 1% of patients with lung adenocarcinoma carry A-Raf-S214 mutations, with S214C and S214F being the most predominant variants (37).

In BRAF wild-type Langerhans cell histiocytosis, a somatic compound mutation in A-Raf [p.Q347_A348del + p.F351L (c.1044_1049del + c.1053C>G)] was detected within the kinase domain. Mutant A-Raf exhibits markedly elevated MEK phosphorylation activity compared with the wild-type form, thereby enhancing *in vitro* cellular transformation and hyperactivating the MAPK/ERK pathway (38).

A-Raf mutations are detected in 11% of intrahepatic cholangiocarcinoma (iCCA) samples, including the A-Raf-N217I (c.650A>T; p.Asn217Ile) and A-Raf-G322S (c.964G>A; p.Gly322Ser) mutations. Both are heterozygous, cell-activating mutations that are frequently observed in tumor cells and most commonly occur in moderately to poorly differentiated peripheral-type iCCA adenocarcinomas. By relieving autoinhibition and enhancing dimerization, these mutations drive constitutive MAPK pathway activation (39). The A-Raf-R362H mutation simultaneously inhibits the dimerization of A-Raf with itself (homodimerization) and with CRAF (heterodimerization) and reduces the catalytic activity of A-Raf, resulting in a kinase-deficient mutant. Compared with wild-type A-Raf, A-Raf-R362H entirely loses its capacity to suppress ERBB3, resulting in abrogation of the tumor-suppressive function. This leads to hyperactivation of the ERBB3-Akt pathway in lung cancer, markedly enhancing cellular invasion and metastasis and worsening the clinical prognosis (Table I) (40).

7. Effects of upstream regulatory factors on A-Raf function

hnRNPH, as a splicing factor, serves an important role in the normal expression of mature A-Raf mRNA. The oncogene c-Myc is frequently upregulated in cancer types including colorectal cancer, small cell lung cancer and nasopharyngeal carcinoma, regulating the alternative splicing of A-Raf mRNA by upregulating hnRNPH. This results in increased production of full-length A-Raf (oncogenic) and decreased production of the truncated form A-Rafshort (tumor-suppressive). Full-length A-Raf binds to MST2, thereby inhibiting apoptosis and promoting cell survival, whereas A-Rafshort lacks kinase activity and is unable to bind to MST2. By altering the balance between these two isoforms, c-Myc controls ERK pathway activity and influences the risk of cellular carcinogenesis (19,41). Upon activation of microglia by lipopolysaccharide, a marked shift in selective polyadenylation sites of A-Raf occurs, compared with the resting state. Alternative polyadenylation generates different A-Raf transcript isoforms with long and short 3' untranslated regions. The short A-Raf mRNA isoform encodes a dominant-negative protein lacking kinase activity, which is highly expressed in resting microglia and limits inflammation by inhibiting the Ras-ERK pathway (42).

Eukaryotic translation initiation factor 5A-1 (EIF5A1) is located upstream of A-Raf and markedly upregulates A-Raf protein expression. A-Raf serves as a central signaling hub for EIF5A1-mediated trophoblast function. By integrating post-transcriptional mRNA regulation with the integrin/ERK signaling pathway, A-Raf maintains the cell migration and invasion capabilities required for placental development (43). Downregulation of A-Raf may contribute to recurrent miscarriage (43). TH1 is a specific interacting partner of A-Raf (44) and selectively downregulates A-Raf. Upon binding, TH1 directly inhibits A-Raf phosphorylation and its capacity to phosphorylate the downstream substrate MEK. The TH1-A-Raf interaction suppresses the expression of the downstream cell cycle-associated gene cyclin D1, thereby reducing the proportion of cells in the S phase and attenuating the rate of cell proliferation (17). Among the Raf family members, A-Raf exhibits the lowest sensitivity to oncogenic Ras and tyrosine kinases and A-Raf activation is the weakest (45).

In mouse insulinoma cells, pyruvate kinase M1 directly binds to A-Raf and upregulates its activity, which in turn specifically activates the MEK1/ERK signaling pathway and attenuates endoplasmic reticulum stress-induced apoptosis (46,47). The regulatory subunit casein kinase 2 β subunit (CK2β) of the protein kinase casein kinase 2 is a specific activator of A-Raf capable of selectively activating A-Raf. Co-expression of CK2β and A-Raf markedly enhances A-Raf-mediated MEK phosphorylation (by 10-fold), establishing CK2β as a potent activator of the A-Raf pathway (48).

Treatment of cultured ventricular cardiomyocytes with classic myocardial hypertrophy agonists, such as endothelin-1, angiotensin II and α-adrenergic agonists, has been shown to markedly increase A-Raf kinase activity in a time-dependent manner. This activation in turn stimulates the downstream MEK-ERK pathway and contributes to the regulation of pathological processes, including cardiomyocyte hypertrophy and phenotypic remodeling (49). IL-3 markedly upregulates A-Raf kinase activity. This activation is entirely dependent on the PI3K pathway. IL-3-activated A-Raf directly phosphorylates and activates downstream MEK, thereby initiating the ERK signaling cascade. The MEK-ERK signaling pathway is the core pathway through which IL-3 regulates hematopoietic cell proliferation and survival. The activation of A-Raf maintains normal proliferation and survival of hematopoietic cells (50).

Table I. Mutations in A-Raf.

Mutation type	Mutated site	Domain	Molecular mechanisms	Biological effects	(Refs.)
Missense mutation	A-Raf-S214p	CR2	Eliminates phosphorylation sites, weakens 14-3-3 binding and lifts self-inhibition	Primarily causes lymphoendothelial dysplasia and lymphatic malformations	(36)
Missense mutation	A-Raf-S214c	CR2	Eliminates phosphorylation sites, weakens 14-3-3 binding and lifts self-inhibition	Drives the malignant progression of lung adenocarcinoma	(37)
Multiplex mutation	p.Q347_A348del + p.F351L (c.1044_1049del + c.1053C>G)	C-terminal kinase domain	Stabilizes the active conformation of A-Raf and enhances its ability to form homodimers and heterodimers with CRAF	Promotes abnormal proliferation, differentiation and migration of Langerhans cells, inducing malignant transformation	(38)
Missense mutation	A-Raf-N217I	CR2	Relieves the self-inhibitory conformation of the N-terminus of A-Raf, thereby reducing 14-3-3 binding	Promotes abnormal proliferation and loss of cell cycle control in cholangiocarcinoma cells, inhibits apoptosis and enhances tumor cell survival and increases the potential for invasion and metastasis	(39)
Missense mutation	A-Raf-G322S	A-Raf kinase domain (TKD)	Locks A-Raf in an active, open conformation to enhance its ability to recognize and phosphorylate MEK1/2	Promotes abnormal proliferation and loss of cell cycle control in cholangiocarcinoma cells, inhibits apoptosis, enhances tumor cell survival and increases the potential for invasion and metastasis	(39)
Missense mutation	A-Raf-R362H	TKD	Completely inhibits kinase activity and lifts the transcriptional repression of ERBB3	Converts A-Raf from a lung cancer metastasis inhibitor to a pro-metastasis factor, enhancing tumor cell survival, EMT and invasive and metastatic capabilities	(39)

CR2, conserved region 2; EMT, epithelial-mesenchymal transition; ERBB3, erb-b2 receptor tyrosine kinase 3; TKD, tyrosine kinase domain.

8. A-Raf-associated functional models

A-Raf functions as a signaling hub through a kinase-independent pathway. There is key cross-regulation between the RAF signaling pathway and the MST2 tumor suppressor pathway. A-Raf inhibits MST2 by binding to and sequestering it, independent of its own kinase activity. During epithelial cell differentiation, the function of the A-Raf-MST2 complex is regulated by subcellular compartmentalization. In proliferating cells of the squamous epithelial basement membrane and in tumor cells, A-Raf localizes to mitochondria, thereby efficiently sequestering and inhibiting MST2. By contrast, in normal squamous epithelial cells, A-Raf is distributed at the cell membrane, where the sequestration of MST2 is inhibited (18). A-Raf can also upregulate and activate RAS. Upon binding to RAS, A-Raf competitively displaces the GTPase-activating protein NF1, thereby antagonizing NF1-mediated inhibition of RAS. This reduced ERK-dependent inhibition of RAS and increased RAS-GTP. This mechanism regulates the duration and downstream effects of receptor tyrosine kinase (RTK)-induced RAS activation, where activated RAS-GTP binds to A-Raf, induces its homo- and heterodimerization, activates the kinase activity of A-Raf and thereby promotes cell proliferation, sustaining signal output in RTK-dependent tumor cells. Consequently, in human lung cancer types harboring EGFR mutations, A-Raf amplification has been associated with acquired resistance to EGFR inhibitors (51).

As a metabolic regulator, A-Raf has been implicated in the maintenance of cellular energy homeostasis and the modulation of anabolic pathways (24). A-Raf and M2-PK are interacting proteins. A-Raf induces M2-PK dimerization, thereby inactivating the enzyme and reducing the efficiency of glucose-to-lactate conversion. In immortalized NIH3T3 fibroblasts, however, the oncogenic mutant form of A-Raf increases the proportion of highly active M2-PK tetramers, thereby enhancing the energy yield of the glycolytic pathway (24).

9. A-Raf-associated treatment

A-Raf can serve as a therapeutic target to reverse drug resistance and inhibit Ras. The highly disordered N-terminal sequence of A-Raf drives protein self-assembly, leading to the formation of A-Raf-Ras granule aggregates. This structure markedly inhibits Ras GTPase-activating protein-mediated negative regulation of NF1 membrane recruitment, thereby sustaining Ras activation. A-Raf is a key determinant of the sensitivity of A-Raf-mutant tumors to RAF inhibitors; knockout of A-Raf has been shown to enhance the sensitivity of RAS-mutant cells to RAF inhibitors (34). The use of pan-RAF inhibitors in tumors such as non-small cell lung cancer induces signaling reprogramming in tumor cells, activating A-Raf-mediated compensatory bypass pathways and promoting the formation of A-Raf-kinase suppressor of Ras 1 complexes, which sustain MAPK signaling and contribute to the development of drug resistance. Concurrent inhibition of RAS or MEK can effectively block this resistance mechanism (52).

A-Raf mutations serve as predictive biomarkers for disease diagnosis and therapeutic intervention. A patient with advanced lung adenocarcinoma treated with oral sorafenib achieved near-complete clinical and radiological remission sustained for up to 5 years. Genomic and transcriptomic sequencing was performed on primary tumor tissue samples and corresponding normal control samples from patients. Somatic S214C mutations were detected in the majority of tumor tissues, whereas none were identified in the normal control samples. These findings indicate that mutated A-Raf functions as an oncogenic driver in lung adenocarcinoma and may serve as a predictive biomarker for sorafenib efficacy (37).

A-Raf in combination with acrylic acid-polyethylene glycol-N-hydroxysuccinimide (AC-PEG-NHS) exerts synergistic effects on bone repair. A-Raf mediates osteoblast differentiation induced by mechanical stretch and is involved in osteogenesis and bone mass maintenance. Cartilage organoids cultured with AC-PEG-NHS can regenerate neocartilage with a gene expression profile highly similar to that of normal healthy cartilage. Co-repair mediated by osteoblasts and chondrocytes recapitulates the native anatomical structure and achieves complementary mechanical properties, thereby facilitating regulation of the bone microenvironment and shortening of the repair cycle (53).

10. Challenges and prospects

As an intracellular protein kinase, A-Raf serves a central role in the regulation of the cell cycle and apoptosis, the transport and metabolism of substances, organismal development, bone mass maintenance and tumor progression. Genetic variations, such as mutations in the A-Raf gene, affect A-Raf function. Furthermore, current research indicates that the function of A-Raf is regulated by a number of factors, including intracellular signaling molecules (3,18,28), proteins involved in RNA metabolism regulation (19,20,31,41), bioactive substances (46-50) and A-Raf-binding proteins (17,24,25,44). However, the detailed mechanisms by which A-Raf influences the cell cycle, apoptosis and cell proliferation require further elucidation. However, A-Raf inhibition may promote tumor metastasis and drug resistance through CRAF/BRAF compensatory activation, RAS bypass or upregulation of the ERBB3-Akt pathway. Given that the MAPK pathway regulates bone growth and the GH/IGF-1 axis, its long-term inhibition may result in growth restriction, delayed bone age and abnormal reproductive development, raising ethical concerns regarding irreversible long-term hazards in pediatric patients. Through in-depth research, it may be possible to identify patient populations sensitive to A-Raf-targeted therapy, develop specific inhibitors against particular A-Raf mutations and explore combination treatment strategies that integrate A-Raf targeting with immunotherapy, chemotherapy and radiotherapy. Considering the important function of A-Raf in tumor progression, it is further hypothesized that this approach not only reduces the risk of tumor recurrence and metastasis but also improves patient prognosis, offering novel treatment options for cancer.

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Availability of data and materials

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Authors' contributions

MY wrote the manuscript. MC, JW and YLi collected the related papers and helped revise the manuscript. YZ, YLei and CL designed and revised the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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