



REVIEW

New druggable targets in the Ras pathway?

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Ras proteins are key elements in the regulation of cellular proliferation, differentiation and survival. Mutational activation of Ras or of components of its effector pathways are detected in one-third of human cancers and are essential for the genesis and maintenance of the tumoral phenotype. Research efforts have been dedicated to the development of therapeutic agents that inhibit aberrant Ras signals and, subsequently, tumor progression. However, many of these initiatives have proven less successful than expected. This review summarizes the current status of developments in Ras research, the challenges that have arisen during preclinical and clinical stages, and how novel approaches to targeting Ras pathways have introduced new strategies toward the development of antitumoral agents that are alternative or complementary to those currently in use. These new approaches would be aimed at disrupting key protein-protein interactions that are essential for the conveyance of Ras aberrant signals or would be directed against new proteins recently demonstrated to be critical participants in Ras-regulated pathways.

Keywords Antitumor therapy, B-Raf, Cancer, ERK, MAPK, MEK, Ras

Introduction

Three decades have passed since seminal discoveries demonstrated a causal link between the *ras* genes harbored in murine sarcoma retroviruses and cancer pathogenesis [1]. In this time, the *RAS* gene products, the GTPases, H-Ras, N-Ras and K-Ras 4B/4A, have been recognized as key signal transducers. The mechanisms whereby Ras is regulated by guanine-nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) have been elucidated. In addition, the constituents of the main effector pathways through which Ras relays its signals to the interior of the cell have been identified and the distinct actions of Ras signals in different cellular microenvironments are being investigated [2,3]. The unraveling of these biochemical milestones has progressed in parallel to the acquisition of a broad knowledge of the role of Ras in cancer. Since the early 1980s, during which *HRAS* was identified as the first human oncogene and its activating mutations were defined, mutant alleles of the three *RAS* genes have been detected in many human cancers. Analysis of more than 40,000 tumor samples indicates an activating mutation rate of 22, 8.2 and 3.7% for *KRAS*, *NRAS* and *HRAS*, respectively [4]. If cases in which mutational activation is detected (in most cases in a non-overlapping occurrence) in components of Ras effector pathways are considered, namely *BRAF* (22%) and p110 α *PI3K* (12%), the proportion of human neoplasia exhibiting a

hyperactive Ras-related pathway is greater than 50% [5,6]. In addition, a vast body of data has been gathered substantiating the importance of Ras signals in cancer initiation and progression. Activated mutants of Ras or of its downstream effectors have been demonstrated to induce malignant transformation in many cell types, as a result of unregulated proliferation, differentiation or survival [1]. Pharmacological and genetic inhibition of Ras signals have demonstrated the role of this protein in the activation and maintenance of the transformed phenotype [2,4]. In addition, sophisticated animal models have supported the importance of Ras and its downstream routes for tumorigenesis *in vivo* [7].

As a result, it is not surprising that Ras has attracted enormous attention, both in academia and industry, for its potential as a target in cancer therapy. Large amounts of research have been dedicated to strategies directed at curtailing Ras aberrant signals as a means of halting tumor progression. Most of these initiatives have been aimed at either inactivating Ras or inhibiting the activity of some of its downstream kinases. The results have been mixed. The attempts to inactivate oncogenic Ras have been mostly unsuccessful [8-10]. In the case of the approaches toward inhibiting downstream kinases, mostly directed against Raf and MEK family kinases, several generations of inhibitors have been under investigation. Some inhibitors have advanced

through clinical trials, but most have been hampered by poor clinical efficacy and/or undesired toxic effects [11].

Research to identify alternative approaches to target Ras signals to enable the generation of more efficient and less toxic inhibitors is ongoing. New data have unveiled a plethora of proteins and processes that have critical regulatory roles in Ras signaling. Moreover, novel functional interactions have been identified, introducing new players into Ras-regulated pathways that could provide potential new targets for therapeutic intervention. This review presents an overview of these novel findings that could provide new approaches for interfering with Ras signals.

Old drugs for old concepts

Since it was determined that amino acid substitutions at codons 12, 13 and 61 impaired Ras GTPase activity, making it unresponsive to GAPs, substantial efforts were devoted to identify approaches to restore Ras enzymatic activity; thus far, this research has been unsuccessful [12,13]. As an alternative strategy, Ras access to the plasma membrane (PM), which is essential for the biological activity of Ras [14], was inhibited using farnesyltransferase inhibitors. These studies yielded positive results in mouse models of H-Ras; however, these results were not replicated in clinical trials [4], likely because K-Ras and N-Ras, unlike H-Ras, are also modified by geranylgeranylation, a process that increases in activity when farnesylation is blocked (for a review, see reference [15]). Combined inhibition of farnesylation and geranylgeranylation demonstrated high toxicity in preclinical studies, and was not considered to be a viable option [9]. Other attempts to inhibit Ras activity included the blockade of Ras expression using antisense oligonucleotides [16], but this strategy has not been successful because of the high level of specificity of these molecules and the difficulty of delivery to the target tumors [17]. Another approach has been the use of inhibitors of prenylated protein methyltransferase (PPMTase), the enzyme that methylates Ras proteins. PPMTases inhibit Ras-dependent cell growth by an unknown mechanism that is probably unrelated to the inhibition of Ras methylation [18]. The most potent of these inhibitors, *S-trans,trans*-farnesylthiosalicylic acid (FTS) (salirasib, Concordia Pharmaceuticals), has undergone phase I trials for solid tumors with promising results [19] (Table 1); however, Ras remains a difficult molecule to target pharmacologically.

Raf inhibitors

The difficulties experienced with developing Ras inhibitors have resulted in a shift in focus to several downstream kinases of Ras pathways, particularly those kinases that form the cascade leading to the activation of ERK MAP kinases [4] (Figure 1). The Raf kinase family (ie, ARaf, BRaf and cRaf) has emerged as an appealing target, after the discovery of activating mutations in *BRAF* in 60% of melanomas, 40% of thyroid tumors, and

20% of colorectal and ovarian tumors [20,21]. In addition to sorafenib (Nexavar) [22,23], which is approved for the treatment of hepatocellular and renal cell carcinoma, three Raf inhibitors are being evaluated in clinical trials: RAF-265 (Novartis; ClinicalTrials.gov identifier: NCT00304525); vemurafenib (Plexxikon/F Hoffman-La Roche) [24] and XL-281 (Bristol-Myers Squibb; NCT00451880 and NCT01086267; for reviews, see references [11,25]) (Table 1). These inhibitors function as ATP-competitive analogs that, by definition, exhibit some degree of unspecificity. Thus, doubts remain as to whether the antitumoral effects of these agents are a result of off-target effects or their anti-Raf activity. Such is the case for sorafenib, which also inhibits VEGF-2, VEGF-3 and PDGF β ; the anti-oncogenic properties of this agent are independent of its inhibitory effect on BRaf [26]. Another caveat is that tumors tend to acquire resistance to these inhibitors, probably as a result of 'gatekeeper' mutations. For example, resistance to vemurafenib is developed in a median time of 8 to 9 months of treatment [27]. Of major concern are recent discoveries demonstrating that Raf inhibitors can have opposite effects to those intended, depending on the cellular context. In tumors harboring oncogenic K-Ras mutations, Raf inhibitors promote ERK activation in a Ras-dependent manner, with subsequent stimulation of tumor growth [28,29]. Similarly, a dead-kinase mutant of BRaf and oncogenic K-Ras cooperate to induce melanoma in mice [30]. These results highlight the need for screening patients for BRaf and Ras mutations in order to distinguish those patients likely to respond from those in which anti-Raf therapies could be harmful. Importantly, these adverse interactions also reduce the therapeutic options available to treat the large number of tumors harboring Ras mutations.

MEK kinase inhibitors

Inhibitors of MEK kinases (MEK1 and MEK2) are also available as a therapeutic option. Unlike Raf inhibitors, MEK kinase inhibitors are not ATP mimetics and, consequently, exhibit high specificity. The mode of action of MEK kinase inhibitors results from binding to a unique inhibitor-binding pocket, locking the kinase in a closed, inactive form [31]. PD-184352 (Pfizer) [32,33], PD-0325901 (Pfizer) [25,34], AZD-6244 (AstraZeneca) [35] and XL-518 (Genentech/Exelixis; NCT00467779) have undergone clinical trials (Table 1). PD-184352 demonstrated insufficient clinical activity, but its positive safety profile has encouraged the development of derivatives [32]. The development of PD-0325901 was discontinued because of toxicity concerns [25,36]. AZD-6244 has completed phase I trials in patients with advanced cancer [37] and is in phase II trials in combination with other chemotherapeutic agents, including sorafenib and PI3K inhibitors, following promising results in mouse models [38,39]. XL-518 has been reported to inhibit ERK1/2 activation in a preclinical xenograft model and to be well tolerated in a phase I trial [40]. The development of resistance is also a concern for MEK inhibitors. Screening of tumors

Table 1. Drugs targeting Ras-regulated pathways.

Compound (developing company)	Target	Tumor type	Highest development status	Reference
Salirasib (Concordia Pharmaceuticals)	Ras	Solid tumor NSCLC	Phase II	[19]
Sorafenib	Raf, PDGFR β , VEGFR-2/3, c-KIT and FLT-3	Advanced renal cell carcinoma Advanced hepatocellular carcinoma	Launched	[22,23]
vemurafenib (Plexikon/ F Hoffman-La Roche)	Mutant BRaf	Melanoma Colorectal tumor Solid tumor	Phase III	[98]
XL-281 (Bristol-Myers Squibb)	All Raf isoforms and mutant BRaf	Advanced solid tumor	Phase I	NCT00451880 and NCT01086267
RAF-265 (Novartis)	Raf and VEGFR	Advanced melanoma	Phase I	NCT00304525
PD-184352	MEK1/2	Colon cancer Pancreatic cancer Breast cancer NSCLC	Discontinued	[32,33]
PD-0325901	MEK1/2	Advanced solid tumor Breast cancer Colon cancer Melanoma	Discontinued	[11,34]
AZD-6244 (AstraZeneca)	MEK1/2	Advanced melanoma Biliary cancer Pancreatic cancer NSCLC Advanced colon cancer Hepatocellular carcinoma Thyroid tumor Solid tumor	Phase II	[35]
XL-518 (Genentech/Exelixis)	MEK1/2	Solid tumor	Phase I	NCT00467779
GDC-0941 (Genentech)	Class I PI3K	Advanced solid tumors Breast tumors Non-Hodgkin's lymphoma	Phase I	NCT00876122, NCT00960960 and NCT00996892
MK-2206 (Merck & Co)	AKT	Advanced solid tumors	Phase II	NCT01071018 and NCT01186705
Perifosine (Keryx Biopharmaceuticals/ AEterna Zentaris/Handok Pharmaceuticals)	AKT	Advanced solid tumors Melanoma Multiple myeloma Renal cell carcinoma Leukemia Sarcoma	Phase III	[52] NCT01002248
Everolimus	mTORC1	Soft-tissue and bone sarcoma Advanced solid tumors Brain tumor Head and neck cancer Breast cancer Prostate cancer	Launched	[56,99]
Temsirolimus	mTORC1	Advanced renal cell carcinoma Advanced solid tumors Myeloma NSCLC Endometrial cancer	Launched	[53,54,100]

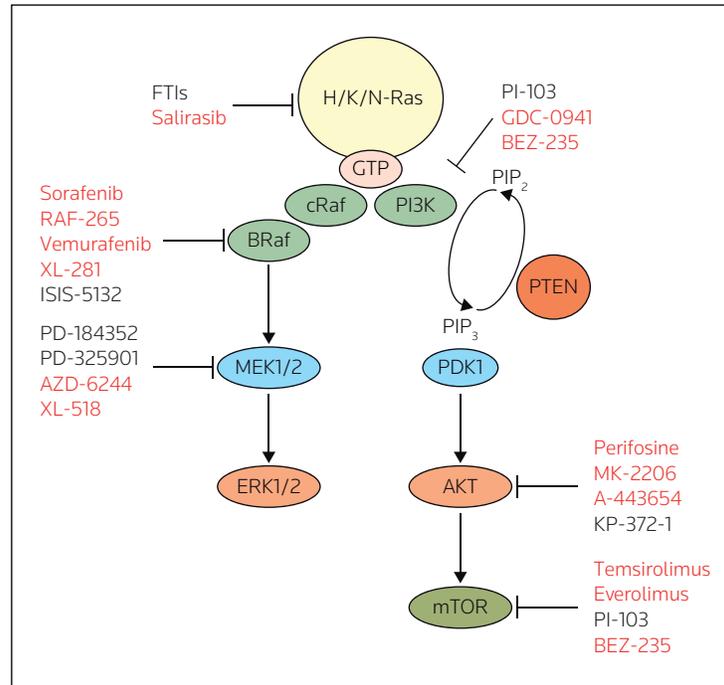
from relapsed patients following AZD-6244 treatment detected mutations in MEK that conferred resistance to the inhibitor [41]. Resistance also can develop in the absence of mutations in MEK itself, probably as a result of alterations in other key regulatory molecules. For example, K-Ras activation has been demonstrated to confer resistance to PD-184352 [42]. Notably, mutations

activating the PI3K pathway are a major resistance mechanism for MEK inhibitors in tumors harboring wild-type MEK [43].

PI3K inhibitors

The available pharmacological agents that target Ras-mediated signals are not restricted to the Raf-ERK

Figure 1. 'Classical' therapeutic targets in Ras pathways.



A simplified representation of the Ras effector pathways harboring components with enzymatic activities that are targeted for antitumor therapy. Therapeutic agents in black have been discontinued.

FTI Farnesyltransferase inhibitor

pathway. The PI3K pathway has also been the subject of significant research. Ras-GTP interacts with the p110 α and p110 β catalytic subunits of PI3K, activating a pathway that generates a strong anti-apoptotic and pro-proliferative signal (Figure 1). The PI3K pathway is negatively regulated by the phosphatase PTEN [44]. Different components of the PI3K signaling pathway are deregulated in cancer. The loss of PTEN functions occurs in 30 to 40% of tumors [5], resulting in the maintenance of the downstream kinase AKT in a hyperactive state. Gain-of-function mutations have also been detected in p110 α , and amplifications are frequent in the gene encoding p110 β [45]. Finally, somatic mutations and amplifications of the *AKT* family genes have also been reported [46]. Unlike the Raf pathway, overlapping mutations in different components of the PI3K pathway can occur depending on the tumor type [47]: in endometrial cancers, Ras and PI3K mutations are mutually exclusive, suggesting that p110 α is not necessary for the initiation of these tumors. Conversely, concomitant mutations of Ras and p110 α are observed in 7% of colorectal cancers, indicating that these oncogenes synergize to confer a selective advantage in these cells [48]. The co-existence of such mutations is likely to be helpful to classify patients for treatment. A major concern of targeting the PI3K pathway alone is that this approach may not be sufficient to halt tumor progression. However, the use of these drugs in combination with treatments against other pathways could prove beneficial for patients with cancer.

Several inhibitors against different components of the PI3K pathway, such as AKT and mTOR, are under evaluation. LY-294002 and wortmannin were the first PI3K inhibitors to be used in preclinical studies. These inhibitors were highly unspecific and very toxic in animals models, but derivatives are being developed, some of which are in early clinical trials (eg, SF-1126 [Semafore Pharmaceuticals] and PX-866 [Oncothyreon]) [49,50]. The observation that the PI3K inhibitor PI-103 also inhibited mTOR [51] has led to the development of dual PI3K-mTOR inhibitors. The rationale behind this approach is that inhibiting both components concomitantly could have a stronger antitumoral effect, though concerns for potential severe side effects also exist [49]. Nevertheless, derivatives of PI-103, such as GDC-0941 (Genentech; NCT00876109; phase I), and other dual inhibitors are undergoing clinical trials [44]. With respect to AKT family kinases, two types of inhibitors have been developed: ATP-competitive inhibitors and non-catalytic inhibitors. Most of the ATP-mimetic drugs can inhibit all AKT isoforms, as well as other members of the AGC kinase family. For this reason, isoform-specific inhibitors are being developed. The non-catalytic inhibitors function by masking the pleckstrin homology domain, thereby preventing AKT binding to the membrane. These non-catalytic inhibitors include perifosine (Keryx Biopharmaceuticals/AEterna Zentaris/Handok Pharmaceuticals) [52] and MK-2206 (Merck & Co; NCT01071018), which are in phase III and phase II clinical trials, respectively. Finally, mTOR inhibitors are available as cancer therapy. Rapamycin is approved for

the treatment of renal carcinoma [44], and several derivatives of this inhibitor have been developed and approved, such as temsirolimus [53,54] and everolimus [55,56]. One limitation of mTOR inhibitors is that these compounds can trigger the activation of PI3K through the inhibition of a negative feedback loop [57]. However, this effect may be overcome by the use of dual PI3K-mTOR inhibitors.

While more of these 'classical' inhibitors of Ras pathways are expected to be delivered in the near future, the question arises whether new approaches to Ras-regulated pathways can be used to develop alternative types of drugs to inhibit aberrant signaling.

The Ras-ERK pathway: Targeting dimerization

The characteristic of ERKs to dimerize in response to stimulation is well known [58]; however, the biochemical and biological significance of ERK dimerization were unknown until recently, when it was demonstrated that ERK dimers are formed using scaffold proteins as dimerization platforms [59]. These scaffold-dimer complexes are critical for relaying the cytoplasmic signals of ERK, by managing the interaction of ERKs with their cytoplasmic substrates. In contrast, activation of the nuclear substrates of ERK is mostly undertaken by ERK monomers [59]. Importantly, the inhibition of the cytoplasmic component of ERK, by preventing ERK dimerization, is sufficient to abrogate cellular transformation and proliferation, as well as tumor formation in xenografts of lung, colorectal and bladder carcinoma cells in mice [59].

Dimerization seems to be a common theme in the ERK cascade. BRaf and cRaf heterodimerize in a Ras-dependent manner following stimulation [60,61], whereas oncogenic mutants dimerize constitutively. This process requires the participation of the protein 14-3-3 and is essential for cRaf transactivation by BRaf [62]. Importantly, mutations that prevent dimerization of *Drosophila* Raf also impair its catalytic function [63]. Similarly, BRaf that cannot dimerize as a result of similar mutations is incapable of transactivating cRaf, stimulating ERK phosphorylation [28] and inducing transformation [64]. MEK family kinases also dimerize. MEK1 and MEK2 form stable heterodimers not regulated by growth factor stimulation. These heterodimers are critical for fine-tuning the amplitude and duration of ERK activation, by a mechanism that entails negative feedback regulation by ERK via phosphorylation of MEK1. In the absence of phosphorylated MEK1, heterodimer formation is prevented, and MEK2 phosphorylation and ERK activation are prolonged [65].

These findings highlight that the pathway leading to ERK activation involves much more than phosphorylation; critical protein-protein interactions must occur to ensure the propagation of the ERK signals. Some of these interactions offer, at least conceptually, attractive targets for future antitumoral drugs. The demonstration that

inhibiting ERK dimerization by genetic means is sufficient for halting tumoral cell proliferation [59] and recent findings suggesting that inhibiting ERK dimerization potentiates the apoptotic effects of drugs such as cisplatin, paclitaxel and doxorubicin [Crespo P: unpublished data], has led to the screening for compounds that can prevent ERK dimerization. This effort has led to the identification of several compounds that prevent ERK dimerization that are undergoing further evaluation [Crespo P: unpublished data].

Similarly, Raf heterodimerization also demonstrates potential as a target for therapeutic intervention. As demonstrated for *Drosophila* Raf [63] and mammalian BRaf-cRaf dimers [28], preventing the formation of Raf dimers diminishes ERK signaling significantly. Strategies for impeding the association between BRaf and cRaf could be aimed directly at the dimerization interface, but also at the 14-3-3 binding sites, an essential interaction for dimerization to occur [62,64,66]. Unlike Raf and ERK dimers, MEK heterodimers appear not to be a suitable therapeutic target because blocking MEK1-MEK2 dimerization would result in enhancing ERK activation [65]. Importantly, dimerization interfaces and other protein-protein interaction motifs are probably unique regarding their molecular structure and interactions. Thus, targeting these structures could yield drugs with higher specificity, and subsequently less undesired, off-target, secondary effects, compared with those compounds resulting from conventional strategies directed at inhibiting the enzymatic activities of kinases.

Spatial regulators as therapeutic targets

Recent discoveries have established the concept that Ras signals are the sum of multiple, site-specified sub-signals [67]. Conceptually, searching for compounds that selectively block Ras sub-signals essential for tumor progression should produce drugs with reduced side effects, compared with compounds that block Ras signaling completely. It is known that within the PM, Ras is present at distinct microdomains [68]. In addition, Ras is also present in different endomembranes (for a review, see reference [3]). At these sites, Ras is subject to site-specific control mechanisms undertaken by various regulatory proteins (for an extensive review, see reference [69]). Recent studies have demonstrated that cellular transformation can be prevented/reverted by the inhibition of specific, location-defined sub-signals. For example, transformation by oncogenes such as v-Src and Sis can be prevented by the inhibition of Ras signals generated by lipid rafts or disordered membrane [70]. Annexin A6, an ancillary protein that facilitates Ras inactivation via p120 GAP, suppresses Ras-induced transformation by recruiting p120 GAP specifically to non-raft PM microdomains [71]. Inhibiting Galectin-1, a protein essential to stabilize active H-Ras in non-raft PM 'nanoclusters', dislodges H-Ras from these structures and prevents fibroblast transformation [72]. These data illustrate that it is not necessary to suppress Ras signaling completely

in order to obtain growth/transformation-suppressive responses. Thus, although conceptual, strategies directed at modulating the functions of some of these site-specific regulators could be a valid therapeutic option in the future.

The same concept is applicable to events downstream from Ras. ERKs are found in the cytoplasm in unstimulated cells; an important fraction of ERKs migrates to the nucleus upon phosphorylation, where the ERKs perform essential functions [73]. However, the extranuclear component of ERK is as important; approximately half of the ~180 proteins identified as ERK substrates are non-nuclear proteins [74]. The nuclear and cytoplasmic components of ERKs are potential targets for antineoplastic therapy. Ample data demonstrate that sequestering ERKs at the cytoplasm, thereby impeding ERK nuclear signals, is sufficient for abrogating growth or provoking apoptosis in tumor cells [75-77]. The nucleo-cytoplasmic shuttling of ERKs is finely regulated. For efficient nuclear translocation, ERKs require direct interaction with the nuclear pore complex, and the participation of nuclear shuttles and a nuclear translocation signal, Ser-Pro-Ser, within the 'insert' domain, that is phosphorylated upon stimulation, promoting nuclear translocation [78]. Conceptually, drugs aimed at masking this short sequence could represent an option to stop the nuclear translocation of ERK. As mentioned previously, inhibiting the cytoplasmic component of ERK by disrupting its dimerization is sufficient to prevent tumor progression [59]. Thus, the blockade of either of these subcellular components may be a valid strategy for future therapeutic intervention.

The amplitude and intensity of ERK signals are regulated by scaffold proteins that assemble the components of the signaling cascade into a complex whereby signal optimization is achieved [79]. Scaffolds also play important roles in the spatial selectivity of ERK, operating as transmitters of Ras signals, originating at different microenvironments, to specific ERK substrates [80]. Distinct scaffolds appear to operate in different subcellular localizations: KSR1 regulates ERKs signals at PM cholesterol-rich domains [81]; MP-1 at endosomes [82]; Sef at the Golgi complex [83]; paxillin at focal adhesions [84]; and β -arrestins in clathrin-coated pits [85]. Scaffold proteins are candidates with enormous potential to become site-specific therapeutic targets. Some of these proteins, such as KSR1, have no known function other than regulating ERKs, so no off-target effects are expected. This specificity could make KSR1 an ideal target for intervention. Indeed, mice deficient in KSR1 develop normally, but are resistant to tumor development [86]. Unfortunately, much important structural information related to ERK has not yet been elucidated, including how MEK and ERK dock onto KSR1. These sites harbor the potential to become hotspots for the design of drugs aimed at disrupting ERK signals through competitive binding to scaffolds.

The Ras apoptotic route as a therapeutic target

It is well known that oncogenic Ras can trigger apoptosis in different cell types. Recent findings involve RASSF family proteins as critical pro-apoptotic effectors [87,88]. In response to Ras activation, members of this family, such as RASSF1, NORE1 and RASSF2, activate the pro-apoptotic kinases MST1 and MST2, engaging a pathway through which cell survival is regulated (Figure 2) [89-91]. Conversely, cRaf inhibits MST2 in a kinase-independent manner [92,93]. Thus, MST2 association with RASSF family proteins promotes apoptosis, whereas the interaction of MST2 with cRaf prevents it, resulting in aberrant growth. Although many aspects of MST1/2 regulation remain unknown, targeting MST1/2 could be of use in the treatment of tumors harboring Ras, and possibly BRAf, mutations. The development of inhibitors for the interaction between cRaf and MST2, thereby shifting the balance toward MST2 association with RASSF, should direct tumor cells to apoptosis.

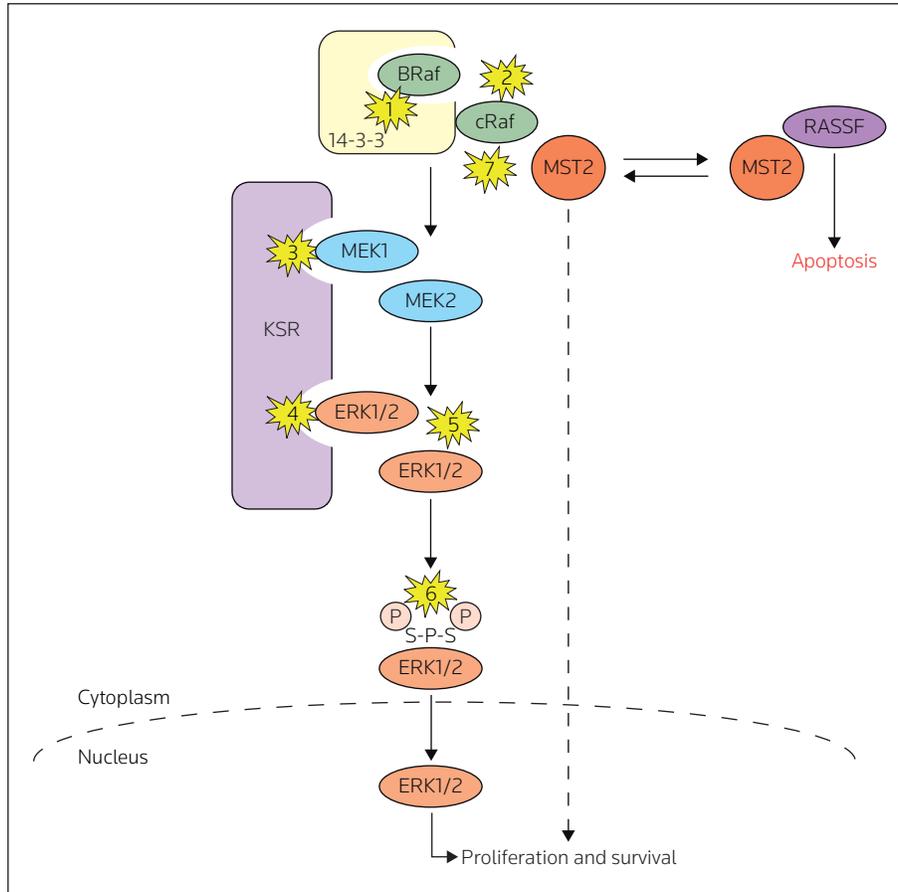
Alternative strategies to treat Ras tumors

Despite significant research efforts and investments devoted to the development of drugs aimed at targets within the Ras pathways, there has been limited success thus far. For this reason, new approaches are necessary. Recently, the concept of 'non-oncogene addiction' has been proposed, based on the observations that certain normal genes are necessary for the maintenance of the tumoral phenotype [94]. These genes can be directly regulated by an oncogene, but can also act in parallel pathways and, therefore, not appear as obvious candidates for pharmacological intervention. If these genes are inhibited, there is a synthetic lethality effect resulting in the activation of senescence or apoptotic responses, causing tumor regression [94]. This concept has been used to identify genes necessary for the maintenance of 'Ras-addicted' tumors. Using RNAi screening, PLK1, STK33, SYK, RON, TBK and integrin β 6 have been identified as essential for the progression of tumors harboring oncogenic Ras [95-97]. In the near future, more of these genes will be identified, some of which will have the potential to be exploited for the development of new antineoplastic therapies.

Conclusion

The knowledge acquired during the past three decades regarding Ras and its related pathways has led to significant advances in tumor treatment. Unfortunately, many of the expectations for Ras pathway-targeted drugs have not been fulfilled. High toxicity and resistance acquisition have hampered many of the drugs developed to date. While more of these 'classical' inhibitors are to be expected, recent findings in the Ras field have revealed new players and novel functional interactions that provide an alternative approach to target Ras signals by focusing on protein-protein interactions rather than enzymatic activities. These novel findings could introduce a new era in drug discovery and in the

Figure 2. Novel strategies for targeting Ras pathways.



A simplified model of Ras effector pathways harboring potential hot spots for therapeutic intervention, based on disrupting protein-protein interactions rather than enzymatic activities, is presented. The potential strategies include: (1) Inhibition of cRaf transactivation by B-Raf via disrupting the interaction between the 14-3-3 and B-Raf proteins; (2) inhibitors of B-Raf/cRaf heterodimerization; (3) and (4) inhibition of MEK1/2 and ERK1/2 interaction with their scaffolds; (5) inhibition of ERK1/2 dimerization; (6) regulation of ERK nuclear translocation; and (7) inhibition of cRaf/MST2 interaction.

development of new types of drugs for the treatment of tumors with mutations in the Ras signaling pathways.

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