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ERKs in Cancer: Friends or Foes?

Xavier Deschênes-Simard^{1,3}, Filippos Kottakis³, Sylvain Meloche², and Gerardo Ferbeyre¹

Abstract

The extracellular signal-regulated kinase ERK1 and ERK2 (ERK1/2) cascade regulates a variety of cellular processes by phosphorylating multiple target proteins. The outcome of its activation ranges from stimulation of cell survival and proliferation to triggering tumor suppressor responses such as cell differentiation, cell senescence, and apoptosis. This pathway is intimately linked to cancer as several of its upstream activators are frequently mutated in human disease and are shown to accelerate tumorigenesis when engineered in the mouse genome. However, measurement of activated ERKs in human cancers or mouse models does not always support a role in tumorigenesis, and data consistent with a role in tumor suppression have been reported as well. The intensity of ERK signaling, negative feedback loops that regulate the pathway, and cross-talks with other signaling pathways, seem to be of primary importance in determining the final cellular outcome. Cell senescence, a putative tumor-suppression mechanism, depends on high-intensity ERK signals that trigger phosphorylation-dependent protein degradation of multiple proteins required for cell-cycle progression. This response may be circumvented during carcinogenesis by a variety of mechanisms, some of them yet to be discovered, which in essence turn ERK functions from tumor suppression to tumor promotion. The use of pharmacologic inhibitors targeting this pathway must be carefully evaluated so they are applied to cases in which ERKs are mainly oncogenic. *Cancer Res*; 74(2); 1–8. ©2014 AACR.

Introduction

The extracellular signal-regulated kinases (ERK1/2; MAPK3/1) are ubiquitous regulators of multiple cellular processes such as proliferation, differentiation, survival, and transformation. These kinases are the last components of a signaling module composed of the small GTPase RAS and the protein kinases RAF and MEK1/2 (MAP2K1/2; ref. 1). With an overall mutation incidence of up to 30% in human cancer, mutant RAS is among the most common human oncogenes (2). RAF mutations are also frequent, particularly in melanoma (3), but MAP-ERK kinase (MEK) mutations are rare and ERK mutations have never been reported as drivers in human cancers. Nevertheless, current thinking proposes that both RAS and RAF oncogenes promote human cancers by activating the ERK kinases (2, 4). Consistent with this idea is the fact that ERK kinases positively regulate the cell cycle by increasing the availability of building blocks for cell growth (5), by stimulating the cyclin-dependent kinase (CDK)-cyclin complexes required for cell-cycle progres-

sion (6), and by preventing cell death (7). In addition, deregulated nuclear accumulation of activated ERKs (pERK) can lead to genomic instability and subsequent tumor progression (8). On the other hand, recent results indicate that the ERK kinases may trigger tumor suppressor pathways as well (9, 10) and that this activity depends on the strength of their activation (9). Hence, the role of ERK kinases in human cancers appears to be context dependent and more complex than originally suspected, reflecting its involvement in both oncogenesis and tumor suppression.

Clinical studies indicate a variable association between ERK activation and human cancers, consistent with either an oncogenic or a tumor-suppressing role. Consequently, ERK activation in human cancers has been linked to either good or bad prognosis (Supplementary Data). Otherwise, ERKs might be required in cancer cells for proliferation and survival, but their activation could be transient because of the activation of negative feedback mechanisms still not understood (11). Therefore, it is unclear whether sustained ERK hyperphosphorylation is an obligate prerequisite of cancer initiation or progression despite activated oncogenes upstream of the pathway. There are also confounding issues in clinical studies that use immunohistochemistry (IHC) to determine the status of ERK signaling. Detection of phosphoepitopes depends on the quality of the antibody chosen and the time taken to fix tissues after obtaining them from patients (12). Furthermore, the most critical issue is the definition of high and low staining. Most studies use internal scoring systems and report relative levels. Only a few reports include downstream components of the pathway such as ERK targets or gene expression signatures that could give a better assessment of signaling strength. We are thus left with only a qualitative assessment of ERK signaling

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and therefore we can hardly make conclusions about outputs of this pathway that depend on signaling strength.

In mice, ERK kinases are essential for survival, complicating the efforts of testing their role in tumorigenesis (13). Interestingly, in KRAS-driven lung cancer, decreasing the total ERK amount by genetic ablation of ERK1 or ERK2 had no significant effect on tumor development. However, eliminating both ERK isoforms abrogated tumor development, suggesting that a minimal amount of ERKs is still required (13). The authors also evaluated the effect of total ERK deletion in the whole animal and observed rapid death. Consequently, can the need for the ERK kinases in tumors be explained by a general requirement for cell viability, or rather by a specific role in neoplastic transformation?

Lessons from ERK Pathway Inhibitors

Given the difficulties in evaluating the role of ERK kinases in human and mouse cancers using IHC data, studies using inhibitors of the pathway may shed additional light into their function. Many studies have been conducted with BRAF and MEK inhibitors. It is usually assumed that RAF, MEK, and ERK act in a linear signaling pathway, so the results of using these inhibitors are interpreted as an inhibition of the ERK kinases. However, there is evidence that RAF (14) and MEK kinases (15) exert ERK-independent functions and therefore results obtained with the use of these inhibitors may not exclusively reflect the involvement of ERK.

MEK inhibitors (AZD6244 or selumetinib and PD0325901) reduced tumor formation in mouse xenograft models (16) and achieved stable disease in phase II trials in patients with advanced cancer (17, 18). However, they were not superior to conventional cytotoxic chemotherapy in patients with advanced melanoma (19) and failed to improve liver cancer patients' outcomes despite reducing pERK levels (20). Intriguingly, using anchorage-independent growth as an *in vitro* surrogate of transformation, ERK activation was shown to be a poor predictor of sensitivity to MEK inhibitors (21, 22). Several studies reported that inhibition of ERKs by MEK inhibitors may be compensated by phosphoinositide 3-kinase (PI3K)/AKT pathway activation (23) and numerous mechanisms have been proposed to explain this effect. These include the relief of an ERK-dependent negative feedback loop on receptor tyrosine kinase (RTK) activation (24, 25) and a dynamic reprogramming of the kinome due to MYC degradation and subsequent induction of RTKs (26). Thus, combining MEK inhibitors with AKT pathway inhibitors could improve anticancer activity, a concept that has been validated in murine models (27).

In patients with melanoma, the BRAF inhibitor vemurafenib (PLX4032) induces partial or complete tumor regression, and this correlates with inhibition of cytoplasmic ERK activation (28). However, patients develop resistance to vemurafenib within 6 months of treatment, and analysis of their tumors revealed changes that reactivate the ERK pathway (29) or increase signaling through RTKs in an ERK-independent manner (30). In cells expressing BRAFV600E, negative feedback mechanisms activated by pERKs suppress RTK-dependent RAS activation. The SPRY proteins are in part

responsible for this process (31). BRAF inhibitors relieve this feedback mechanism and thereby improve the cell response to RTK ligands and signaling through RAS and CRAF (RAF1), possibly explaining the ERK reactivation (30, 31). On the basis of the latter hypothesis, clinical trials that combine BRAF and MEK inhibitors in patients with melanoma have been designed to overcome the development of resistance to BRAF inhibitors (32). This combination proved to be moderately better than individual treatments (32, 33), supporting the proposition that the rebound in pERKs is most likely related to BRAF inhibitor resistance. However, it was not demonstrated that MEK inhibitors acted specifically on the ERK kinases and some results indicate that MEK may have other targets (15, 34). Another important concern is that the combination of a BRAF inhibitor with a MEK inhibitor improves survival but does not cure patients. Moreover, in patients with melanoma who were not selected according to BRAF status, MEK inhibitors did not improve progression-free survival and induced significant toxicity (19). In the context of a combination therapy, the acquisition of simultaneous BRAF and MEK inhibitor resistance is a plausible explanation for a treatment failure. Reactivation of ERKs by COT-mediated MEK-independent ERK phosphorylation is a candidate mechanism allowing this dual resistance (35). This hypothesis raised the interest in developing ERK inhibitors to circumvent resistance to BRAF and MEK inhibitors. Although *in vivo* and long-term experiments are still needed to reach conclusions on their value, selective pyrimidylpyrrole ERK inhibitors have been shown to inhibit cell proliferation of some MEK inhibitor-resistant cancer cell lines (36). However, the failure of BRAF/MEK inhibitors in therapies is also consistent with the idea that ERK-independent pathways (30) may contribute to tumor progression in melanoma. Melanoma cell lines resistant to BRAF inhibitors are often resistant to MEK inhibitors but sensitive to inhibition of AKT. These cells also display persistent AKT activation (37), indicating again that combining AKT pathway inhibitors with BRAF/MEK inhibitors may achieve better clinical response.

Some patients with melanoma treated with the BRAF inhibitor vemurafenib develop new nonmelanoma skin cancers in the first few weeks after the start of therapy, and many of these lesions display mutations in the RAS oncogene (38). Also, at least one case of leukemia with RAS mutations has been reported in patients with melanoma undergoing BRAF inhibitor treatment (39). Vemurafenib probably stimulates the growth of preexisting lesions rather than causing them directly, and that is likely why this phenomenon is not observed in every patient. In a mouse model of skin carcinogenesis known to induce RAS mutations, vemurafenib reduced tumor latency (38). Accordingly, the authors of the study proposed that the treatment led to a paradoxical increase in ERK activation and accelerated tumor progression (38). A proposed mechanism to explain this paradox is an unexpected ability of the inhibited BRAF isoform to heterodimerize with CRAF to increase its activation by the RAS oncogene (40). However, because treatment with these inhibitors also reduced ERK activation (28), it remains a challenge to determine the context and kinetics of ERK inhibition and ERK activation by BRAF inhibitors.

Models Emerging from Genetically Engineered Mice

Studies in genetically engineered mouse models for activated oncogenes upstream of the ERK/mitogen-activated protein kinase (MAPK) pathway have provided conflicting insights about the role of ERK kinases in cancer, just like the clinical data. KRAS is the most frequent mutational target upstream of ERK kinases in human cancers, and diverse mouse models have been generated to study its functions (reviewed in ref. 41). Mice with mutant HRAS, NRAS, and RAF were also developed and provided similar observations (Supplementary Data).

An increase of pERKs was demonstrated in multiple KRAS-driven benign neoplasms, each showing a correlation with elevated levels of pERKs and markers of cellular senescence, such as p16^{ink4a} (CDKN2A/p16), p19^{Arf} (CDKN2A/p19), p53 (TP53), and promyelocytic leukemia protein (PML; ref. 42). These observations suggest that the ERK kinases engage tumor-suppressor genes, and that abrogation of these genes must be performed for tumor initiation from cells with elevated pERK levels (Fig. 1A). This model shows agreement with the observations reported for colonic serrated adenocarcinoma in which KRAS induces oncogene-induced senescence (OIS) and the ERK/MAPK pathway but not the other effectors of RAS, such as the AKT and RAL pathways. The loss of Ink4a in this context circumvented OIS and allowed the hyperplastic lesions to progress into serrated cancers (43).

On the other hand, even if the activation of the KRAS oncogene in the lung was shown sufficient to promote pulmonary adenocarcinoma (44), no IHC evidence of increased pERK levels was initially found during cancer initiation or in most tumors (45). Only a subset of late-stage tumors in the context of a p53 loss showed activation of these kinases (46). In this context, elevated ERK phosphorylation tightly correlated with p19^{Arf} upregulation, a potent activator of the tumor suppressor p53 in mice (47). Likely, p53 loss abrogated this ERK-mediated tumor suppression axis. Intriguingly, CRAF, but not BRAF, was found critical for lung cancer initiation in KRASG12D mice. Nonetheless, lack of CRAF was shown to have no significant effect on pERK levels, whereas BRAF ablation does (48). It is possible that CRAF regulates an ERK-independent pathway essential for tumor initiation, possibly, the reported inhibition of the ROK α kinase pathway in skin carcinogenesis (14). In other malignant lesions or hyperproliferative disorders, such as adenocarcinomas of the colon and a myeloproliferative disorder resembling chronic myelogenous leukemia, the pERKs were again not significantly upregulated by oncogenic KRAS (49–51). These reports suggest that negative regulators of the ERK/MAPK pathway may be involved to limit its activation by oncogenic KRAS in certain cellular contexts and raise questions about the importance of the pathway in these cancers. As observed in mouse embryonic fibroblasts (MEF) derived from mice expressing endogenous levels of KRASG12D (45), such inhibition of the pathway may circumvent high ERK signaling-induced senescence to allow tumor initiation (Fig. 1B).

In pancreatic cancers, the role of the ERK/MAPK pathway is still controversial. Elevated levels of pERKs were found in pancreatic premalignant lesions and in pancreatic ductal

adenocarcinoma (PDAC) cells (52, 53). The MEK inhibitor PD325901 inhibited tumor growth in orthotopic xenografts of mouse PDA-derived cell lines, suggesting a role for ERK in tumor progression (54). In addition, the genetic ablation of the EGF receptor (EGFR) was shown to compromise pancreatic tumorigenesis in mice expressing oncogenic KRAS (55, 56). This dependency can be explained by induction of a robust RAS–RAF–MEK–ERK pathway activity, further suggesting its requirement for transformation. However, treatment with the EGFR inhibitor erlotinib in this context resulted in strong inactivation of the PI3K/AKT pathway (55). Furthermore, another study published simultaneously has revealed that either treatment with erlotinib or genetic ablation of EGFR in pancreas expressing KRASG12D caused a robust inhibition of the PI3K/AKT pathway, but had no effect on pERK levels (56). In this regard, the PI3K/AKT pathway, but not CRAF, was shown to be genetically essential for KRAS-induced PDAC initiation, thereby suggesting that the activation of AKT rather than a hyperactivation of the ERK kinases is critical for tumor initiation in this cancer (57). Overall, these observations suggest that the PI3K/AKT pathway could play a critical role in RAS-driven cancers and raises questions about the relative contribution of a hyperactivation of the ERK pathway in this process.

OIS markers have been found in preneoplasms during the early stages of PDAC tumorigenesis (58, 59). The activation of the PI3K/AKT pathway was shown to antagonize this state and resulted in rapid development of PDAC (60, 61), providing a mechanistic explanation for its critical role in PDAC onset. Although the capacity of AKT signaling to inhibit senescence in lung premalignant lesions has not yet been demonstrated, accumulating observations suggest such a mechanism. Premalignant lung adenomas were shown to be positive for senescence markers (62), and activation of AKT by PTEN loss accelerates KRAS-initiated tumorigenesis (63). Furthermore, a point mutation in the p110 α catalytic PI3K subunit (PIK3CA) introduced to inhibit its interaction with RAS in a KRAS-driven lung adenocarcinoma model abrogated signaling to AKT, but not ERKs, and strongly reduced tumor formation (64). The critical role of AKT signaling in KRAS-driven lung cancer onset was then confirmed by genetic deletion of the p85 PI3K regulatory subunit (65). The molecular mechanisms explaining the capacity of the PI3K/AKT pathway to circumvent tumor suppression will need further investigation, but may include inactivation of tumor-suppressor mechanisms induced by high ERK signaling or inhibition of the ERK/MAPK pathway (Fig. 1A and B).

The ERK pathway has been also studied in mouse models for loss of function of negative regulators of the pathway. The genetic ablation of both *Spry1* and *Spry2* alleles, which are negative regulators of RTK signaling, leads to the expected increase in pERK levels, but no significant regulation of AKT signaling was observed in the prostate. This context induced frequent ductal hyperplasia, occasionally progressing into low-grade prostatic intraepithelial neoplasia (PIN) lesions (66). These lesions were previously shown to display markers of senescence (67). However, when the *Spry* alleles are deleted in the context of heterozygosity for a

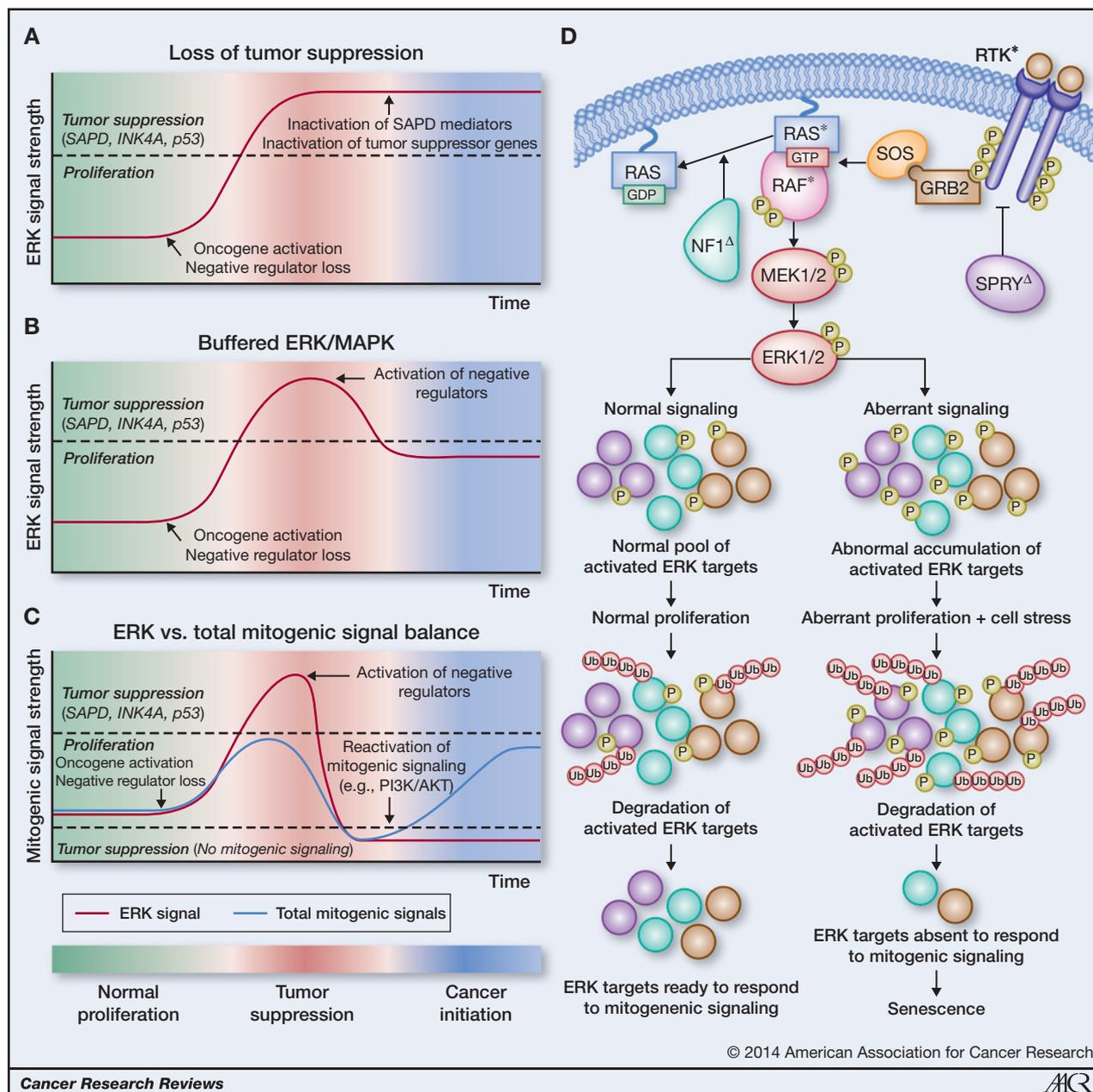


Figure 1. Modeling ERK signaling intensity in tumor suppression and cancer initiation. **A**, high ERK/MAPK pathway activity mediates tumor suppression by inducing the senescence-associated protein degradation (SAPD) of targets of ERK signaling (direct or indirect). Loss-of-function of tumor suppressors or SAPD effectors (i.e., E3 ligases) may allow bypass of senescence and cancer initiation. **B**, high ERK signaling induces tumor suppression as described before. The activation of negative regulators of the ERK/MAPK pathway buffer its activation below the threshold required for tumor suppression. **C**, after activation of tumor suppression as previously, hyperactivated ERK induces strong negative feedback mechanisms, reducing mitogenic signaling below the minimal threshold required for proliferation. Reactivation of other mitogenic signaling pathways may thus enable proliferation and cancer initiation. A moderate activation of the ERK/MAPK pathway is not excluded. **D**, simplified model of ERK-mediated SAPD. Aberrant ERK activity leads to the direct or indirect phosphorylation of a larger fraction of targets, whereas normal signaling engages the phosphorylation of a minor fraction. Some of these targets with sustained phosphorylation undergo phosphorylation-dependent ubiquitination and proteasome-dependent degradation. *, oncogenic activation reported; Δ , negative regulator loss reported; Ub, ubiquitin; P, phosphorylation.

Pten null allele, the development of high-grade PINs is promoted and evidence of neoplastic invasion is observed (66). Interestingly, the loss of Sprouty function cooperates with the loss of one allele of the *Pten* gene to promote AKT activation. Reciprocally, overexpression of *Spry2* in *Pten* null

animals inhibited the hyperactivation of AKT and suppressed *Pten* ablation-driven tumorigenesis (66). Overall, this study may suggest that a loss of function of Sprouty promotes prostatic cancer initiation by the release of a negative regulation on the AKT pathway rather than (or in

addition to) a negative feedback on the ERK/MAPK pathway. Such a conclusion suggests again that the PI3K/AKT pathway could circumvent ERK-induced OIS. Loss of function of another negative regulator of the ERK/MAPK pathway, the RAS-GAP neurofibromatosis type I (NF1), was also studied in mice and showed dissimilarities according to the ERK status in tumorigenesis. A lack of NF1 has been shown to drive benign lesions, such as plexiform neurofibromas (68) and gastric hyperplasia, in conjunction with hyperactivation of RAS and the ERKs (69). Inactivation of tumor-suppressor genes in addition to *Nf1* inactivation leads to diverse frank malignant tumors (68, 70, 71), including astrocytomas (72). These observations are consistent with a model in which abrogation of tumor-suppressor genes is a prerequisite for malignant lesion initiation with elevated pERK levels (Fig. 1A).

An important limitation with the classic genetically engineered mouse models is that the genetic modifications are applied to the whole animal, the whole organ, or all the cells of a specific lineage. Such an approach greatly increases the penetrance because the chances that a cell with the prerequisite to transform is being affected by the desired genetic modification are high. These tools are helpful to study the initiation of malignancy from the emerging lesions and then cancer progression and maintenance. However, those models do not allow an accurate study of the cell of origin. Evidence suggests tissue- and context-specific differences in ERK regulation, thereby reflecting a different role of the pathway in the context of oncogenic RAS and likely explaining divergent propensities to undergo neoplastic transformation (42). If high ERK activity has a ubiquitous role in promoting tumor suppression, we can speculate that tissues with a higher subpopulation of cells with increased capacity to buffer the activation of the ERK/MAPK pathway could develop malignant tumors more frequently in the context of spontaneous and random mutations in upstream regulators of the pathway. In mice engineered to allow spontaneous expression of mutant KRAS in different tissues, mainly lung tumors were observed. Intriguingly, tumors of the pancreas and colon, in which RAS mutations are frequent in humans, were not observed (73). It is possible that these differences are due to differences between humans and mice, but we suggest that the mouse lung may contain more transformation-permissive cells with the capacity to reduce ERK activation. Such a speculation is consistent with the reported low pERK levels found during KRAS-driven initiation of lung adenocarcinoma in mice (45).

ERKs, Protein Degradation, and Tumor Suppression

Tumor suppressor pathways have necessarily evolved to respond to oncogenic threats. Consequently, these pathways must be intrinsically wired to respond to aberrant growth factor signaling. The ERK kinases are at the center of multiple signaling pathways activated by growth factor receptors and other proliferation stimuli. Hence, tumor-suppressor pathways might have evolved to recognize aberrant ERK signaling. Regarding the response to ERK signaling intensity, studies in

genetically engineered mouse models proposed so far two models in which high ERK activity mediates senescence and tumors arise after inactivating the senescence program (Fig. 1A) or downregulation of pro-senescent high ERK activation (Fig. 1B). However, in some contexts, senescent cells and lesions correlate with extinction of the ERK/MAPK pathway (74, 75). These studies have shown that the mechanisms involve negative feedbacks affecting upstream activators of RAS, and thereby inhibiting signaling by the latter, including the PI3K and the RAL pathways. It is thus likely that senescence in this case is maintained by the downregulation of multiple mitogenic signaling pathways and not only the ERK pathway (Fig. 1C).

The ERK kinases transduce their signals by phosphorylating multiple targets, which for the most part, execute cell growth signals. During RAS-induced senescence, aberrant signaling through ERK kinases led to the proteasome-dependent degradation of multiple phosphoproteins required for cell growth and cell-cycle progression (9). The degradation of these phosphorylated proteins is suspected to contribute to cellular stresses associated with senescence, including mitochondrial dysfunction, telomere dysfunction, and DNA damage. This process was named senescence-associated protein degradation or SAPD (Fig. 1D). SAPD depended on ERK activation and was also observed during senescence triggered by short telomeres. Although SAPD may require specific E3 ligases activated by the senescence program, it is the aberrant phosphorylation triggered by high ERK signaling that renders multiple proteins sensitive to depletion by this mechanism. Lowering the strength of ERK activity with pharmacologic inhibitors of the pathway or RNA interference (RNAi) prevented protein degradation and allowed RAS-expressing cells to become malignant (9). The mechanistic details of this tumor-suppression pathway will be the focus of future studies, but it can be anticipated that it involves novel functions of the ubiquitin-dependent proteasome pathway (9) and a tumor-suppressive role for E3 ligases mediating the recognition of phosphorylated proteins. Of note, PML, which is important for OIS, plays a role in the degradation of MYC (76) and could be implicated in the degradation of other targets of the SAPD.

Recognizing the tumor-suppressive function of the ERK kinases and the characterization of the molecular mechanisms implicated will allow a better assessment of the role of the ERK kinases in human tumors. Cancers may avoid the barrier for proliferation provided by the SAPD in diverse manners. In some situations, the SAPD mechanisms and the tumor suppressors activated as a consequence of the stress generated by ERK signaling are disabled, allowing cells with high ERK levels to become malignant (Fig. 1A). Other tumors will simply buffer ERK to levels that are not sufficient to trigger tumor suppression. This could be achieved by, for example, overexpression of ERK phosphatases (DUSP; Fig. 1B and Supplementary Data). It has been also observed that activation of the ERK pathway can trigger a negative feedback mechanism on the ERK and other mitogenic pathways preventing cell growth with features of senescence (75). It is not known whether the initial high ERK activity was sufficient to trigger a SAPD process in this context, but tumors could arise in these cells if they

manage to reactivate mitogenic signaling by mutations or epigenetic mechanisms (Fig. 1C).

These models of tumor progression create a framework to target the use of ERK pathway inhibitors. They could be more effective in tumors where the ERK pathway is highly active and the tumor-suppressor responses downstream of ERKs are inactivated during the carcinogenesis process (Fig. 1A). In tumors where ERK activity is low, it is likely that ERK-dependent tumor suppression has not been mutationally inactivated and ERK pathway inhibitors are anticipated to be less effective. In this context, novel anticancer drugs that increase ERK activity over the threshold required to activate tumor suppression could be used. We must keep in mind that tumors resist any attempt to simplify them into clear categories and within the same tumor we can probably find cells with high or low ERK levels with likely different histories of tumor progression. Proper drug combinations should be identified to target these tumors.

Another important active debate about the ERK kinases in cancer is the attribution of nonredundant functions to the two isoforms. Again, conflicting results can be found in the recent literature. Genetic studies in fibroblast cells using quantitative assays of ERK activity have provided compelling evidence for a redundant role of the two ERK isoforms in cell proliferation (77, 78). Conversely, ERK2 but not ERK1 was shown to contribute to cancer progression by promoting epithelial–mesenchymal transition (79) and by inducing the cytokine receptor subunit gp130 (80). Also, ERK2 but not ERK1 has been recently proposed as a critical mediator of RAS-induced senescence in mouse fibroblasts (81), whereas our work in human cells suggested that the two isoforms may contribute to senescence (9). The outcome of inactivating one specific isoform may be influenced by the cellular context and the relative expression of

ERK1 versus ERK2. Senescence depends on the strength of global ERK activation (9), indicating that in tissues where one isoform predominates, its role in senescence will be more important.

In the end, whether ERK signaling is friend or foe will depend on the context, but evolutionary biology indicates that tumor suppression can be adaptive, whereas cancer formation is not for the individual. Therefore, in broad strokes we can theorize that in normal cells with intact tumor suppression modules ERK activity will protect from tumorigenesis, but in altered or mutated cells where some of these modules are disabled the ERKs will promote the cancer phenotype.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: X. Deschênes-Simard, G. Ferbeyre
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X. Deschênes-Simard, G. Ferbeyre
Writing, review, and/or revision of the manuscript: X. Deschênes-Simard, F. Kottakis, S. Meloche, G. Ferbeyre
Study supervision: G. Ferbeyre

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