Sef Downregulation by Ras Causes MEK1/2 to Become Aberrantly Nuclear Localized Leading to Polyploidy and Neoplastic Transformation

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Abstract

Subcellular trafficking of key oncogenic signal pathway components is likely to be crucial for neoplastic transformation, but little is known about how such trafficking processes are spatially controlled. In this study, we show how Ras activation causes aberrant nuclear localization of phosphorylated mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK; MEK) MEK1/2 to drive neoplastic transformation. Phosphorylated MEK1/2 was aberrantly located within the nucleus of primary colorectal tumors and human colon cancer cells, and oncogenic activation of Ras was sufficient to induce nuclear accumulation of phosphorylated MEK1/2 and ERK1/2 in intestinal epithelial cells. Enforced nuclear localization of MEK1 in epithelial cells or fibroblasts was sufficient for hyperactivation of ERK1/2, thereby driving cell proliferation, chromosomal polyploidy, and tumorigenesis. Notably, Ras-induced nuclear accumulation of activated MEK1/2 was reliant on downregulation of the spatial regulator Sef, the repression of which was sufficient to restore normal MEK1/2 localization and a reversal of Ras-induced proliferation and tumorigenesis. Taken together, our findings indicate that Ras-induced downregulation of Sef is an early oncogenic event that contributes to genetic instability and tumor progression by sustaining nuclear ERK1/2 signaling. Cancer Res; 72(3); 626–35. ©2012 AACR.

Introduction

The Ras-dependent Raf/MEK/ERK1/2 mitogen-activated protein (MAP) kinase signaling pathway is a key regulator of mammalian cell proliferation. Studies by various groups have shown the absolute requirement for ERK1/2 signaling in promoting the progression of cells from G0–G1 to S phase (1, 2). Activation of ERK1/2 MAP kinases is associated with the stabilization of c-Myc, the induction of cyclin Ds, and the downregulation of antiproliferative genes throughout G1 phase. ERK1/2 signaling also promotes cell survival in certain cellular contexts (3).

Dysregulation of the Ras/Raf/MEK/ERK1/2 pathway is a common event in human cancer. Activating mutations in RAS and RAF genes are found in approximately 30% and 20% of tumors, respectively (4). Although mutations in MEK1/MEK2 genes are infrequent, numerous studies have documented the hyperactivation of MEK1/2 and ERK1/2 in tumors (5). Carcinogenesis and mouse genetic studies support the view that oncogenic Ras or Raf expression is sufficient to initiate tumor formation in certain tissues, but that additional loss of tumor suppressor genes is required for tumor progression (6–9). In the multistep process of colorectal carcinogenesis, activating mutations of KRAS are acquired at the very early premalignant stage, consistent with a role in tumor initiation and/or progression (10). There is also evidence that activated Ras contributes directly to cancer progression by promoting genomic instability (11, 12). How the ERK1/2 MAP kinase pathway contributes to tumor initiation and progression remains unclear.

The ERK1/2 pathway is activated in response to a wide array of mitogenic factors. Typically, ligand binding to growth factor receptors leads to the activation of Ras, which allows the recruitment of Raf to the membrane and mediates the sequential phosphorylation and activation of Raf, MEK1/MEK2, and ERK1/ERK2 kinases (13). Importantly, the activity of the pathway is modulated by a complex network of regulatory binding proteins and scaffolds, which control the magnitude, duration, and compartmentalization of the signal (13–15). Specifically, the subcellular localization of ERK1/2 plays an important role in dictating the outcome of the cellular processes.
response. In quiescent cells, ERK1/2 are maintained in the cytoplasm through their interaction with MEK1/2, which contain an active nuclear export signal, and a number of other interacting proteins including PEA-15. Sef (similar expression to fgf genes) and tubulin (16). Upon mitogenic stimulation, ERK1/2 dissociate from their cytoplasmic anchors and translocate into the nucleus by facilitated diffusion and by a nuclear translocation signal-dependent mechanism (16). Sequestration of ERK1/2 in the cytoplasm by overexpression of PEA-15 or a catalytically inactive form of MKP-3 impairs nuclear Elk1-dependent transcription and inhibits DNA synthesis (17, 18). Reciprocally, genetic depletion of PEA-15 results in increased ERK1/2 nuclear localization and S phase entry in astrocytes (18). Interestingly, the level of PEA-15 protein expression inversely correlates with the invasive behavior of breast cancer (19), suggesting that mislocalization of ERK1/2 contributes to cancer pathogenesis.

In this study, we found that activated MEK1/2 aberrantly localize to the nucleus in a high percentage of colorectal tumors and in colon cancer cell lines. The nuclear accumulation of MEK1/2 is associated with the Ras-dependent downregulation of the spatial regulator Sef. We show that forced nuclear localization of MEK1, which leads to hyperactive ERK1/2 signaling, is sufficient to induce polypliodization and neoplastic transformation of cells. Our results suggest a potential mechanism by which early oncogenic activation of Ras may contribute to the acquisition of chromosomal instability and tumor progression.

Materials and Methods

Cell culture and retroviral infections

The intestinal epithelial cell line IEC-6 and the human colon adenocarcinoma cell lines HCT116, SW480, and COLO 205 were obtained from American Type Culture Collection and cultured as previously described (20). Human intestinal epithelial cells (HIEC) were kindly provided by J.F. Beaulieu (Université de Sherbrooke) and used as described (21). All cell lines were passaged for less than 3 months after receipt. The identity of the cell lines was regularly checked by morphologic criteria, expression of epithelial markers, and karyotyping. IEC-6 cells were synchronized in G0–G1 by starvation of serum for 30 hours.

The cells were stably infected with retroviral vectors as previously described (22). Transduced cells were selected for 3 days with 4 μg/mL puromycine and polyclonal populations of puromycin-resistant clones were used for all experiments. IEC-6 cell populations expressing H-Ras53V and Sef constructs were obtained by infection with pBabe-HA-H-Ras53V followed by selection with puromycine and subsequent infection with pBabe-HA-Sef.

Cell proliferation, transformation, and anoikis assays

Proliferation rate was measured by the colorimetric MTT assay. Anchorage-independent proliferation was evaluated by culturing cells in soft agar medium. Detachment-induced apoptosis was monitored in tissue culture plates coated with Poly-HEMA (Sigma). The methodology for these assays has been described previously (20).

Results

Aberrant nuclear localization of phosphorylated MEK1/2 and ERK1/2 in colorectal cancer

We analyzed the activation status of MEK1/2 (as monitored by activating loop phosphorylation) by immunohistochemistry in a large colorectal cancer tissue microarray (TMA). We found that 44% of primary colorectal tumors display high levels of cytoplasmic phospho-MEK1/2 staining as compared with 10% of normal tissues (Fig. 1A). Surprisingly, detailed analysis also revealed that 79% of colorectal cancers exhibit aberrant phospho-MEK1/2 staining in the nucleus compared with only 4% of normal colon tissue (Fig. 1B). MEK1/2 are known to shuttle into the nucleus, but are rapidly exported to the cytoplasm by their strong NES, resulting in their nearly exclusive cytoplasmic localization (23–25). The role of MEK1/2 nuclear translocation has remained elusive.

To further document this observation, we examined the subcellular localization of phosphorylated MEK1/2 in a series of human colon carcinoma cell lines and in normal HIECs. The 3 colon cancer lines display constitutive activation of MEK1/2 and ERK1/2 as a result of activating mutations in KRAS (HCT116 and SW480) or BRAF (COLO 205) genes (Fig. 1C). Phospho-MEK1/2 staining was detected almost exclusively in the cytoplasm of HIECs. In sharp contrast, nuclear localization of activated MEK1/2 was observed in 65% to 90% of the cells in tumor cell cultures (Fig. 1D). Analysis of total MEK1 localization also revealed that the protein is excluded from the nuclear compartment of HIECs, but readily accumulates in the nucleus of a significant fraction (16%–18%) of colon cancer cells (Fig. 1D). The subcellular localization of ERK1/2 essentially mirrored that of MEK1/2. Activation loop phosphorylated ERK1/2 were confined to the cytoplasm of HIECs, but accumulated in the nucleus of a majority of cells (76%–83%) in the tested colon carcinoma cell lines (Fig. 1E).

To determine whether oncogenic activation of upstream regulators of the ERK1/2 pathway is sufficient to induce the nuclear mislocalization of MEK1/2, we infected the untransformed rat intestinal epithelial cell line IEC-6 (26) with a retrovirus encoding H-Ras53V. Expression of oncogenic Ras resulted in a marked increase in the activating phosphorylation of MEK1/2 and ERK1/2 (Fig. 2A). Strikingly, hyperactivation of Ras induced the nuclear accumulation of phosphorylated MEK1/2 in 90% of IEC-6 cells, whereas infection with empty vector had no effect (Fig. 2B). Similar observations were made for phosphorylated ERK1/2 (Fig. 2B).

To assess the clinical relevance of nuclear MEK1/2 in colorectal cancer progression, we measured the intensity and localization of phospho-MEK1/2 staining in normal human colonic mucosa and in specimens of hyperplastic, premalignant, and malignant lesions of the colon (Supplementary Fig. S1). Normal mucosa displayed only faint cytoplasmic staining of phospho-MEK1/2 with a few positive nuclei limited to the base of the crypts. In sharp contrast, hyperplastic polyps, adenomatous polyps, villous adenomas, and invasive
These mutations were introduced in the otherwise wild-type MEK1, whereas addition of the myristoylation signal restricts MEK1 plasma membrane retention completely abrogates the transfection phenotype induced by MEK1DD (Fig. 3C). Importantly, ectopic expression of MEK1ΔNES significantly increased the rate of cell proliferation (1.9-fold) as compared with control protein or constitutively active S218D/S222D (DD) mutant. All MEK1 mutants were expressed at comparable levels in IEC-6 populations (Fig. 3B). Immunofluorescence analysis confirmed that disruption of the NES causes the nuclear accumulation of MEK1, whereas addition of the myristoylation signal restricts the protein to the plasma membrane (Fig. 3C). Of note, ectopic expression of MEK1DD also caused the nuclear accumulation of endogenous phosphorylated MEK1/2 and ERK1/2 (Supplementary Fig. S2). We then asked if nuclear targeting of catalytically normal MEK1 modulates ERK1/2 activity. Unexpectedly, we found that ERK1/2 become constitutively active in IEC-6-MEK1ΔNES cells (Fig. 3D).

Phenotypically, we observed that nuclear targeting of MEK1 is sufficient to morphologically transform IEC-6 cells, whereas plasma membrane retention completely abrogates the transformed phenotype induced by MEK1DD (Fig. 3C).

Nuclear accumulation of MEK1 induces morphologic changes and accelerates cell proliferation

To evaluate the biological impact of the aberrant nuclear localization of MEK1/2, we forced the localization of MEK1 in the nucleus by substituting 2 critical leucine residues in the NES by alanine (ΔNES) to prevent nuclear export (24). We constructed another series of MEK1 mutants that contained a N-terminus myristoylation signal (Myr) to target the kinase to the plasma membrane and prevent its nuclear import (Fig. 3A). These mutations were introduced in the otherwise wild-type carcinomas displayed a coarsely granular staining comprising up to 10% of the nuclei located both in the deep and superficial aspects of the lesions. The observation that nuclear staining of phosphorylated MEK1/2 is detected early in premalignant lesions and increases with the malignancy of the tumor suggests that mislocalization of MEK1/2 may contribute to the progression of colorectal cancer.

Figure 1. Phosphorylated MEK1/2 and ERK1/2 aberrantly localize to the nucleus in human colorectal cancer cells. A, TMA analysis of cytoplasmic phospho-MEK1/2 (pMEK1/2) expression in clinical samples of normal (n = 49) and colorectal cancer (n = 406) tissues. Bars indicate the percentage of samples, and colors correspond to the intensity of staining. B, representative pictures of normal colon and colorectal cancer tissues stained for phospho-MEK1/2 expression. Bar graph indicates the percentage of samples displaying nuclear localization of pMEK1/2. Positive nuclear staining corresponds to at least 5% of nuclei of tumor cells showing intense staining. C, immunoblot analysis of pMEK1/2 and phospho-ERK1/2 (pERK1/2) expression in proliferating IECs and colon cancer cell lines. D, immunofluorescence staining of IECs and colon cancer cells for pMEK1/2 (green), MEK1 (red), and DNA (DAPI, blue). Magnification, 100×. Bar, 20 μm. Quantification of pMEK1/2 nuclear localization is graphed as mean ± SEM (n = 5). E, immunofluorescence staining for pERK1/2 (red), ERK1/2 (green), and DNA (blue). Results are graphed as mean ± SEM (n = 4). ***P < 0.001.
vector or wild-type MEK1 (Fig. 3E). This effect was abolished by treatment with the MEK1/2 inhibitor U0126, indicating that the kinase activity of MEK1 is necessary for enhancing cell proliferation. In agreement with this, expression of the nuclear-targeted but catalytically inactive mutant MEK1K97A failed to dysregulate cell proliferation or to induce changes in cell shape (data not shown). Expression of activated MEK1DD markedly accelerated the proliferation of IEC-6 cells and additional disruption of the NES (MEK1DDNES) only slightly augmented this effect (Fig. 3E). Remarkably, targeting of MEK1DD to the plasma membrane completely antagonized its stimulatory effect on cell division. Essentially similar results were obtained with NIH 3T3 fibroblasts (Supplementary Fig. S3A). These findings highlight the importance of the nuclear translocation of MEK1/2 in the regulation of cell proliferation.

Flow cytometry analysis showed that cells expressing nuclear-targeted MEK1NES accumulate in S phase concomitantly with a reduction in the percentage of G1 cells (Fig. 3F and G). Expression of MEK1DD also caused an accumulation of cells in S phase, and this effect was repressed by restraining the kinase to the membrane. Overexpression of wild-type MEK1 or MyrMEK1 had no effect on cell-cycle kinetics. Interestingly, fluorescence-activated cell sorting (FACS) profiles of cells expressing MEK1NES, MEK1DD, and MEK1DDNES also revealed an increase in the percentage of G2/M cells as well as a population of cells with more than 4N DNA content (Fig. 3F and G; Supplementary Fig. S3B and S3C, see below). Thus, persistent localization of MEK1 in the nucleus causes the hyperactivation of ERK1/2, resulting in dysregulation of the cell cycle and increased cell proliferation.

**Nuclear accumulation of MEK1 induces cell transformation**

We next asked whether nuclear targeting of MEK1 is sufficient to transform cells. Ectopic expression of MEK1DD conferred anchorage-independent growth to IEC-6 cells, and this property was completely abolished by restricting its localization to the plasma membrane (Fig. 4A and B). Cells infected with MEK1NES also formed colonies in soft agar, albeit at lower frequency than MEK1DD-expressing cells. Similar results were obtained in NIH 3T3 cells (Supplementary Fig. S3D). In all cases, anchorage-independent growth was prevented by treatment of cells with U0126. Cells expressing MEK1K97AANES also failed to proliferate in soft agar (data not shown). Because anchorage independence is associated with resistance to anoikis, we further examined the capacity of MEK1 mutants to protect IEC-6 cells from detachment-induced cell death. Expression of MEK1DD and MEK1ANES almost completely protected IEC-6 cells from undergoing anoikis (Fig. 4C). In contrast, cells infected with wild-type MEK1 or membrane-targeted mutants rapidly entered into apoptosis in suspension and failed to grow in soft agar.

To analyze the impact of nuclear MEK1 signaling on tumorigenesis in vivo, cells were injected subcutaneously into athymic mice. MEK1DD-expressing IEC-6 cells generated rapidly growing tumors that reached a volume of 1,000 mm³ in only 2 weeks (Fig. 4D). Importantly, cells expressing MEK1ANES also formed tumors of similar size but with a longer latency. Overexpression of wild-type MEK1 never induced the formation of tumors. Thus, nuclear accumulation of MEK1 is sufficient to fully transform immortalized intestinal epithelial cells.

**Nuclear accumulation of MEK1 leads to polyploidization**

Quantitative analysis of flow cytometry profiles (see Fig. 3F) revealed that a significant proportion of asynchronously proliferating MEK1DD- and MEK1ANES-expressing IEC-6 cells exhibit a more than 4N DNA content (Fig. 5A). These polyploid/aneuploid cells are rapidly generated after expression of MEK1ANES, and their percentage increases with the number of cell divisions (Fig. 5B). Notably, infection of IEC-6, MCF-10A, and NIH 3T3 cells with H-RasV12 induces a similar accumulation of polyploid/aneuploid cells (Supplementary Fig. S4). To further characterize the chromosomal aberration induced by nuclear MEK1, we prepared metaphase spreads from IEC-6 cells infected for 14 days with either wild-type MEK1 or MEK1ANES and analyzed their karyotypes. Control IEC-6-MEK1 cells are pseudodiploid or near-diploid with chromosome numbers between 42 and 46 per cell (Fig. 5C; Supplementary Table S1). However, examination of IEC-6-MEK1ANES cells revealed the presence of 2 populations of cells. Although most of the cells were near-diploid (37 to 48 chromosomes), 13% of cells (16 out of 122 Giemsa-stained metaphase spreads) exhibited a chromosome number greater than 90. In all, these data establish a clear link between nuclear MEK1 expression and chromosomal aberrations.

**Mitosis and nuclear accumulation of MEK1**

To determine whether the nuclear accumulation of MEK1 interfered with mitotic progression, we prepared mitotic spreads from MEK1DD- and MEK1ANES-expressing IEC-6 cells (Supplementary Fig. S5). A clear and consistent arrest of metaphase was observed in MEK1DD-expressing cells (Fig. 5E). These results show that nuclear MEK1 interferes with cytokinesis and support the notion that the mitotic arrest of MEK1DD-expressing IEC-6 cells is caused by nuclear MEK1 accumulation.

**Loss of Sef Induces Polyploidy and Tumorigenesis**

The polyploid/aneuploid cells generated by nuclear MEK1 expression were sensitive to inhibition of MEK1/2, indicating that the mitotic arrest observed in these cells is mediated by nuclear MEK1. Therefore, the polyploid/aneuploid cells generated by MEK1 overexpression are highly dependent on the upstream Ras MEK1/2 cascade. This suggests that MEK1 accumulation is an essential event for tumorigenesis induced by MEK1 overexpression. In agreement with this, ectopic expression of MEK1K97AANES failed to transform IEC-6 cells, indicating that the tumor-promoting effects of MEK1 overexpression are dependent on the ability of MEK1 to hyperactivate ERK1/2. These findings highlight the importance of the nuclear targeting of MEK1 in the tumorigenic process.
metaphases) had a near-tetraploid modal number with 81 to 94 chromosomes (Fig. 5C; Supplementary Table S1). Spectral karyotyping analysis confirmed the tetraploid karyotype of a subpopulation of IEC-6-MEK1ΔNES cells with the presence of minute chromosomes in 6 cells and chromosomal deletions in 2 cells (29 metaphases analyzed) but did not reveal other structural rearrangements (Fig. 5D).

A previous study has reported that tetraploid p53−/− mouse mammary epithelial cells produce tumors when injected into nude mice, whereas diploid cells do not (27). To determine whether tetraploidy of IEC-6-MEK1ΔNES cells promotes their tumorigenic potential, diploid and polyploid cells were isolated by flow cytometry and injected immediately into the flanks of athymic mice. Tumors arising from tetraploid cells grew much faster than diploid or unsorted cells, reaching a mean volume of approximately 700 mm³ in 2 weeks (Fig. 5E).

**Downregulation of Sef in colorectal carcinogenesis**

To investigate the mechanism responsible for the abnormal nuclear accumulation of MEK1/2, we first examined the impact of activation loop phosphorylation on the kinetics of nuclear entry. Mimicking the phosphorylation of MEK1 (S218D/S222D) markedly accelerated the nuclear translocation rate of MEK1 in proliferating cells, whereas nuclear uptake of the AA mutant was severely compromised, indicating that phosphorylation of MEK1 is necessary for efficient nuclear import (Supplementary Fig. S5). However, phosphorylation of S218/S222 is not sufficient to relocalize MEK1 predominantly to the nucleus in the absence of LMB. These findings indicate that mechanisms other than activation loop hyperphosphorylation contribute to the nuclear accumulation of MEK1/2 in transformed cells.
One spatial regulator of MEK1/2 is Sef, which was shown to bind MEK1/2 and tether MEK1/2-ERK1/2 complexes to the Golgi apparatus and plasma membrane, thereby blocking ERK1/2 signaling in the nucleus (28). We found that the levels of Sef are markedly downregulated in colon carcinoma cell lines as compared with normal HIECs (Fig. 6A). Oncogenic activation of Ras was sufficient to downregulate the expression of Sef in IEC-6 cells (Fig. 6B). Reciprocally, knockdown of K-Ras by lentiviral shRNAs partially restored the expression of Sef in HCT116 and SW480 cells (Supplementary Fig. S6). We also analyzed the expression of Sef in colon tissue specimens by immunohistochemistry. Normal colonic mucosa displayed a heterogeneous staining pattern for Sef. In positive areas, staining was mainly observed in the cytoplasm of colonic epithelial cells, with some plasma membrane reactivity (Supplementary Fig. S7). Benign adenomatous polyps also showed some cytoplasmic staining albeit at lower levels. However, the epithelial component of most dysplastic villous adenoma and malignant invasive carcinoma specimens failed to express any significant immunoreactivity to Sef under conditions that maintain endothelial staining. Thus, the pattern of Sef expression inversely correlates with phospho-MEK1/2 nuclear staining and tumor malignancy.

Expression of Sef prevents polyploidization and tumorigenesis

To directly test the hypothesis that Sef regulates the intracellular localization of MEK1/2 and the fate of intestinal epithelial cells, we ectopically expressed human Sef at close-to-physiologic levels in colon cancer cell lines by retroviral infection. Quantitative immunoblotting analysis showed that HCT116-Sef and COLO 205-Sef populations express 2.8-fold and 2.5-fold, respectively, higher levels of Sef than HIEC (Fig. 6C). Reexpression of Sef restored the normal cytoplasmic localization of phosphorylated MEK1/2 in HCT116 and COLO 205 cells (Fig. 6D). In infected cells not expressing Sef (low Sef), the abnormal nuclear localization of phospho-MEK1/2 was maintained. Expression of Sef also markedly attenuated the activating phosphorylation of ERK1/2, while preserving the hyperactivation state of MEK1/2 (Fig. 6E). In agreement with this finding, Sef expression slowed down the proliferation rate of HCT116 and COLO 205 cells (Fig. 6F).

We next tested whether expression of Sef is sufficient to revert the polyploidy/aneuploidy and tumorigenesis induced by oncogenic Ras. Reexpression of Sef in Ras-transformed IEC-6 cells induced the cytoplasmic relocalization of phosphorylated MEK1/2 in 94% of the cells, accompanied by a morphologic reversion of the transformed phenotype (Fig. 7A).
Analysis of flow cytometry profiles showed that reexpression of Sef normalizes the cell-cycle distribution of IEC-6-H-RasV12 cells (Fig. 7B). After 12 days, the proportion of cells with more than 4N DNA content was 22.8% in IEC-6-H-RasV12 cells as compared with only 4.6% in cells infected with Sef. To evaluate the impact of Sef on tumor development in vivo, cell populations were injected into athymic mice. IEC-6-H-RasV12 cells formed rapidly growing tumors that reached a volume of approximately 600 mm³ in 21 days (Fig. 7C). Reexpression of Sef dramatically inhibited Ras-induced tumor formation. No tumor was apparent in any of the experimental mice 34 days after transplantation. These results suggest that Sef expression antagonizes Ras-mediated polyploidization and tumorigenesis.

Discussion

Much remains to be learned about the regulation and impact of MEK1/2 subcellular localization. Early studies indicated that MEK1/2 are found exclusively in the cytoplasm during signal transmission. However, later work showed that MEK1/2 translocate to the nucleus but are rapidly exported to the cytoplasm by virtue of a strong NES found at their N-terminus (23–25). The mechanism underlying the nuclear transport of these kinases remains incompletely understood. Previous studies have suggested that nuclear translocation of MEK1/2 is enhanced by mitogenic stimulation, and that phosphorylation of activation loop serines is required for this process (25, 29, 30). In contrast, other studies have failed to observe the nuclear relocalization of MEK1/2 in response to agonists (23, 31). More recently, it has been proposed that phosphorylation of a Thr-Pro-Thr motif in MEK1 regulates its nuclear translocation (32). The results presented here clearly show that nuclear localization of MEK1/2 is a regulated process and that dysregulation occurs in cancer. We found that activated MEK1/2 are aberrantly localized to the nucleus in primary colorectal tumors, and that expression of oncogenic Ras is sufficient to induce the nuclear accumulation of MEK1/2. Our results are consistent with a previous immunohistochemistry study, in which nuclear staining of phospho-MEK1/2 was detected in 55% of colorectal tumors (33).

The nuclear translocation of MEK1/2 is critically dependent on activation loop phosphorylation, which is stimulated by oncogenic Ras. However, the DD mutant of MEK1 fails to accumulate in the nucleus in the absence of LMB, indicating that other mechanisms must contribute to MEK1/2 mislocalization in cancer cells. We show here that oncogenic Ras dramatically downregulates the levels of Sef, a spatial regulator of MEK1/2 nuclear accumulation.
of MEK1/2. Accordingly, the expression of Sef protein is almost undetectable in colon carcinoma lines bearing activating mutations of KRAS, and RNAi silencing of K-Ras restores, at least in part, its expression. Sef was originally identified as a feedback inhibitor of Ras-dependent fibroblast growth factor signaling in zebra fish (34, 35). Subsequent work showed that the inhibitory function of Sef is conserved in vertebrate species. However, the site of action of Sef has been the subject of contradictory reports. Torii and colleagues (28) have shown that Sef binds to MEK1/2 in the cytoplasm and inhibits the dissociation of MEK1/2-ERK1/2 complexes, thereby antagonizing nuclear ERK1/2 signaling. Our current findings further substantiate the idea that Sef is a major spatial regulator of the ERK1/2 pathway. We reveal the inverse relationship that exists between the expression of Sef and the nuclear localization of activated MEK1/2 in both cell lines and clinical tissue samples. Importantly, we show that reexpression of Sef is sufficient to restore the cytoplasmic localization of activated MEK1/2 in Ras-transformed IEC-6 cells and colon carcinoma cells.

Recent studies have shown that Sef levels are downregulated in a majority of human prostate, breast, thyroid, and ovarian carcinomas of intermediate to high grades (36–38). In this study, we found that Sef is strongly expressed in the normal colonic mucosa but that expression in the epithelial compartment progressively declines with the increasing malignancy of colon tumors. Thus, loss of Sef appears to be a common event during epithelial cancer progression. Importantly, our findings provide a molecular framework for understanding the potential role of Sef in tumorigenesis. We show that nuclear mislocalization of MEK1 has a dramatic impact on cellular behavior leading to enhanced proliferation, resistance to apoptosis, polyploidization, and tumorigenesis. Our results extend a previous observation of Fukuda and colleagues (39) who reported that disruption of the NES potentiates the ability of constitutively activated Xenopus MAP kinase kinase to induce morphologic changes and transform rodent fibroblasts in vitro. These findings are relevant to cancer pathogenesis as MEK1/MEK2 are very rarely mutated in epithelial cancers but are activated by upstream oncoproteins and are frequently mislocalized as revealed in this study.

The finding that nuclear targeting of MEK1 leads to the generation of tetraploid cells was unexpected. Accumulating evidence suggests that tetraploidy is a genetically unstable state that can serve as an intermediate on the path to aneuploidy and, ultimately, cancer (40). Indeed, tetraploid p53−/− mammary epithelial cells have an increased frequency of whole chromosome missegregation and are more tumorigenic than their diploid counterparts (27). In agreement with this, we found that tetraploid MEK1ΔNES-expressing IEC-6 cells generate tumors much faster than diploid cells in mice.
An important finding from this study was the observation that nuclear targeting of catalytically normal MEK1 results in constitutive activation of ERK1/2 (Fig. 3D). This suggests that loss of Sef, and the resulting mislocalization of MEK1/2, may contribute to maintain the hyperactive state of ERK1/2 in epithelial tumors by a mechanism independent from mutational activation of upstream regulators. In support of this idea, reexpression of Sef in HCT116 or COLO 205 cells markedly attenuates the activating phosphorylation of ERK1/2 without compromising the hyperphosphorylation of MEK1/2. All these observations lead us to propose the following model to explain the frequent loss of Sef in epithelial tumors and its impact on tumor progression (Supplementary Fig. S8). Mutational activation of Ras causes downregulation of Sef in epithelial cells, leading to nuclear accumulation of activated MEK1/2 and ERK1/2. Nuclear MEK1/2 signaling sustains the hyperactivation state of ERK1/2, by a mechanism that remains to be investigated and synergizes with Ras-dependent activating phosphorylation. Prolonged nuclear ERK1/2 signaling regulates the cell cycle and inhibits apoptosis, leading to polyploidization, CIN, and tumor progression. Collectively, our results suggest a tumor suppressor function for Sef in colorectal cancer. Therefore, preventing the downregulation of Sef would appear as a promising therapeutic scenario in this cancer type.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Gerardo Ferbeyre for reagent, Kim Lévesque for expert animal care and experimentation, Julie Hinsinger for immunohistochemistry, Danielle Gagné for FACS analyses and sorting, Sylvie Lavallée and Claude Rondeau for cytogenetic studies, and Marc Thérien for critical comments.

Grant Support

This work was supported by a grant from the Cancer Research Society to S. Meloche. S. Duhamel is recipient of studentships from the Cole Foundation and CIHR. S. Meloche holds the Canada Research Chair in Cellular Signaling.

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Received June 24, 2011; revised November 4, 2011; accepted December 4, 2011; published online February 1, 2012.

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Cancer Res 2012;72:626-635.