Skin cancer is the most common form of malignancy in the world with epidemic proportions. Identifying the biochemical and molecular mechanisms underlying the events leading to tumors is paramount to designing new and effective treatments that may aid in treating and/or preventing skin cancers. Herein we identify p38 MAPK, along with its positive modulator, Gadd45a, as important regulators of nucleocytoplasmic shuffling of the adenomatous polyposis coli (APC) tumor suppressor. APC normally functions to block β-catenin from promoting cell proliferation and migration/invasion. Keratinocytes lacking proper p38 MAPK activation, either due to lack of Gadd45a or through the use of p38 MAPK-specific inhibitors, are unable to effectively transport APC into the nucleus. We also show that p38 MAPK is able to directly associate with and modulate both casein kinase 2 (CK2) and protein kinase A (PKA), which promote and block APC nuclear import, respectively. We demonstrate that p38 MAPK is able to not only enhance CK2 kinase activity but also suppress PKA kinase activity. Moreover, lack of normal p38 MAPK activity in either Gadd45a-null keratinocytes or in p38 MAPK inhibitor treated keratinocytes leads to decreased CK2 activity and increased PKA activity. In either case, disruption of APC nuclear import results in elevated levels of free cellular, and potentially oncogenic, β-catenin. Numerous tumors, including skin cancers, are associated with high levels of β-catenin, and our data indicate that p38 MAPK signaling, along with Gadd45a, may provide tumor suppressor-like functions in part by promoting APC nuclear localization and effective β-catenin regulation.

Skin cancer is a major public health issue in the developed world and accounts for over 1.3 million new cases each year in the United States alone (1, 2). Recently, we have identified the p38 mitogen-activated protein kinase (MAPK)1 signaling pathway, and one of its positive modulators, Gadd45a, as key components protecting the epidermis against UV radiation-induced tumorigenesis (3). One pathway that is affected by inadequate MAPK signaling is the APC/β-catenin signaling pathway (4). Derepression and/or mutations in either APC complex proteins or its substrate, β-catenin, are linked to numerous cancers, including skin tumors such as basal and squamous cell carcinomas, melanomas, keratoacanthomas, and pilomatrixomas (5–9).

To a great extent, the subcellular localization of β-catenin dictates its function. At the plasma membrane, β-catenin functions as an intracellular component of the adherens junctions complex which is important in cell-cell contact (10–12). Alternatively, unbound, soluble β-catenin may localize to the nucleus, where it functions as a transcription factor in association with T-cell factor (TCF). The β-catenin/TCF bipartite transcription factor is known to target and induce the expression of genes such as c-MYC, cyclin D1, and MMP-7 (matrix metalloproteinase 7), which are involved in promoting cell proliferation, migration, and metastasis (5, 13–18). Consequently, increased levels of β-catenin protein contribute to tumorigenesis and, therefore, require strict modulation.

The primary modulator of β-catenin protein levels is the APC tumor suppressor. APC protein itself will modulate β-catenin protein levels and function on two fronts: in the cytoplasm, APC will function as part of a destruction complex along with several other proteins including Axin, protein phosphatase 2A (PP2A), glycogen synthase kinase 3 (GSK3β), and casein kinase 1 (CK1ε). The primary cytoplasmic function of the APC complex is to phosphorylate β-catenin and direct it for ubiquitination and immediate proteasome degradation (19–22). In the nucleus, APC will function to block the transcriptional activity of β-catenin and also to chaperone β-catenin out of the nucleus and into the cytoplasm either for degradation or for assembly at adherens junctions (23).

In general, proper nucleocytoplasmic transport is an important cellular regulatory mechanism that enables tumor suppressors to adequately perform their essential functions, without which may result in protein mislocalization and consequently loss of function (24, 25). In light of this, it has become increasingly evident that nucleocytoplasmic shuffling of APC is a tightly regulated process (26–31). Normally, APC is predominantly cytoplasmic and its nuclear import is mediated by a combination of NLS-dependent and NLS-independent elements that require the importin-α/β receptor pathway or B56α PP2A subunit chaperone pathway, respectively (30–32). Both APC NLS elements are flanked by casein kinase 2 (CK2) and protein kinase A (PKA) phosphorylation sites that positively and negatively regulate APC nuclear import, respectively (28, 30). While truncated APC mutants lacking both NLS elements

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are able to translocate to the nucleus, studies indicate that both NLS sites are required for optimal nuclear import and that multiple NLS elements increase nuclear import (28).

Similar to CK2, kinases within the MAPK signaling cascade have also been reported to modulate nuclear translocation of numerous targets. For instance, p38 MAPK is known to phosphorylate and contribute to proper nuclear translocation of Smad3/4, estrogen receptor ERα, heat shock protein HSP27, and AP-1 family transcription factors, among others (33–37). Relative to APC/β-catenin, we recently demonstrated that normal sustained MAPK signaling via Gadd45α is necessary for proper APC complex activation and consequently modulation of soluble β-catenin levels (4). Herein we demonstrate that not only is MAPK signaling necessary for proper APC, and subsequently β-catenin, cellular compartmentalization, but that p38 MAPK associates with both CK2 and PKA with diametrically opposing effects, i.e. enhancing CK2 kinase activity while suppressing PKA kinase activity. Inadequate MAPK activity ultimately results in APC cytoplasmic sequestration, along with β-catenin nuclear accumulation and inefficient degradation. These observations provide further evidence that p38 MAPK behaves as a tumor suppressor and that deregulated MAPK signaling may contribute to tumorigenesis by disrupting normal nucleocytoplasmic shuttling regulation.

EXPERIMENTAL PROCEDURES

Cell Culture—Primary mouse keratinocytes were derived from wt and Gadd45α-null newborn mice as described previously (3). In brief, the epidermis is separated from the underlying dermis with Dispase and subsequently trypsinized to obtain single cells. Isolated keratinocytes are in turn seeded onto sterile cover slips and cultured with serum-free medium as specified by the manufacturer (keratinocyte-SFM defined medium, Invitrogen Corp.). SB202190 (Calbiochem) treatment (10 μM) was performed for 8 h prior to either fixation or lysis. β-Gal-NLS1APC and β-Gal-NLS2APC constructs were obtained from Dr. Kristi Neufeld and were transfected into primary mouse keratinocytes with Lipofectamine 2000 according to manufacturer’s specifications (Invitrogen Corp.).

Immunocytochemistry—Cultured keratinocytes were fixed with 4% paraformaldehyde at room temperature for 10 min, followed by permeabilization with 0.2% Triton X-100 for 5 min after washing fixed cells with 1× PBS. Following the permeabilization step, cells were washed with 1× PBS and quenched with fresh 0.1% sodium borohydride for 5 min. Next, cells were blocked with either horse or goat serum for 60 min at room temperature and followed by overnight incubation at 4 °C with the primary antibody diluted with 1% BSA in 1× PBS. Subsequently, cells were washed with 1× PBS and incubated with secondary fluorescent-labeled antibody for 45 min at room temperature. After washing the cells with 1× PBS, coverslips were mounted with VectaShield with DAPI (Vector Laboratories, Inc., catalog number H-1200) and analyzed by immunofluorescence microscopy. Primary antibodies used, with specified dilutions, were as follows: β-catenin, rabbit IgG (1:2000, Cell Signaling Technology); β-Gal, mouse IgG (1:1000, Promega). Secondary antibodies used were as follows: Cy3-conjugated donkey anti-mouse IgG or Cy3-conjugated donkey anti-rabbit IgG (1:2000, Jackson Immunoresearch Laboratories).

Immunoprecipitation—Primary keratinocytes were lysed in buffer composed of: 1% Triton X-100, 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 2.5 mM sodium orthovanadate, 1 mM Na3VO4, 1 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride. PKA was immunoprecipitated from equal amounts of lysate using anti-PKA antibodies (Upstate Biotechnology) followed by incubation with protein A-Sepharose. The immunoprecipitate was washed twice with kinase buffer (Upstate Biotechnology). CK2 immunoprecipitation was performed with agarose conjugated anti-CKIIa antibody and processed as described for PKA above. Washed immunoprecipitates were then utilized for kinase assays as described below.

For co-immunoprecipitation studies, purified bacterially expressed GST-p38 (or GST alone as control) was incubated along with glutathione-Sepharose beads (4 Fast Flow, Amersham Biosciences). Subsequently, GST and GST-p38 samples were washed three times in lysis buffer and incubated with primary keratinocyte lysates at 4 °C. Next, samples were washed five times in lysis buffer and resolved by SDS-PAGE (10% gels).

Immunoblot—Primary mouse keratinocyte cytoplasmic and nuclear fractionations were performed with the NE-PER nuclear and cytoplasmic extraction reagent (NER and CER, respectively) as specified by the manufacturer (Pierce). In brief, 5 × 106 cells were trypsinized (0.05% trypsin, 0.53 mM EDTA) and resuspended in 200 μl of CER I lysis buffer. After a 10-min ice-cold incubation, 11 μl of CER II buffer was added to sample followed by centrifugation to pellet the nuclei. After centrifugation, the supernatant was stored for use as the cytoplasmic fraction, and the nuclei were lysed with 100 μl of NER lysis buffer. SDS-PAGE with either 10% or 12% acrylamide gels (Novex precast gels, Invitrogen Corp.) was performed followed by blotting onto Immobilon™-P transfer membrane (Millipore Corp.). Protein detection by chemiluminescence (Amersham Biosciences) on membranes was conducted according to standard protocols supplied by Cell Signaling Technology. Primary antibodies used were as follows: APC, C-20 clone (1:100, Santa Cruz); P-β-catenin (1:200, Cell Signaling Technology); P-GSK3β (1:200, Cell Signaling Technology); MCM7 (1:500, Santa Cruz); Actin, Ab-1 clone (1:5000, Oncogene). Secondary antibodies used were as follows: anti-rabbit IgG, horseradish peroxidase linked F(ab’)2 fragment (1:2000, Amersham Biosciences) or anti-mouse IgG horseradish peroxidase-linked whole antibody (1:1000, Amersham Biosciences). For kinase assay—Activity PKA and CK2 were performed as specified by Upstate Biotechnology. For PKA, 200 μg of immunoprecipitate in 10-μl volume was added to the following: assay dilution buffer (20 μl), 20 μM cAMP (5 μl), Leu-Arg-Ala-Ser-Leu-Gly (Kemptide) substrate (5 μl), PKC/calmodulin kinase inhibitor (10 μl), and Mg2+/ATP mixture containing γ32P-ATP. Reactions were carried out for 10 min at 30 °C and subsequently 25 μl of sample was blotted onto PVDF phosphocellulose paper. Blotted PKA and CK2 were washed with 0.75% phosphoric acid, dried with acetone, and transferred to scintillation vials for counts. Background counts from reactions minus Kemptide substrate was subtracted from the counts from enzyme samples. The CK2 activity assay was performed in the same manner with the following exceptions: CK2 substrate peptide was used instead of Kemptide, PKA inhibitor was used instead of PKC inhibitor, and 20 μl of 40% trichloroacetic acid was added to reaction mix immediately following 10 min incubation at 30 °C.

RESULTS

p38 MAPK Activity Promotes APC Nuclear Localization—We have previously established that APC complex activation in part requires adequate and sustained p38 MAPK activity (4). Moreover, Gadd45α, a positive modulator of p38 MAPK signaling, associates with and enhances APC complex activity (4). Since proper APC function as a β-catenin modulator necessitates effective shuttling in and out of the nucleus, we investigated the possibility that APC localization may be affected by a disruption in p38 MAPK signaling. Due to the fact that normally APC protein levels are low in wild type (wt) keratinocytes (28),2 to verify the localization status of APC we conducted immunocytochemistry on primary keratinocytes that were transfected with plasmids coding for β-galactosidase linked to either one of the two APC NLS elements (β-Gal-NLS1APC and β-Gal-NLS2APC). The results show that neither Gadd45α-null keratinocytes (Fig. 1A) nor p38 MAPK inhibitor-treated wt keratinocytes (Fig. 1B) demonstrate nuclear localization of β-Gal-NLS1APC. Irrespective of the construct, transfected wt, untreated keratinocyte controls show normally β-Gal localized predominantly in the cytoplasm or perinuclear region of Gadd45α-null and p38 MAPK inhibitor-treated wt keratinocytes, respectively. This phenotypic observation indicates that disruption of p38 MAPK signaling does affect APC subcellular transport and prevents APC from entering the nucleus.

p38 MAPK Signaling Is Necessary for Proper β-Catenin Localization—While there is a clear disruption of normal APC nuclear shuttling in keratinocytes lacking adequate p38 MAPK.

2 J. Hildesheim, Jr., personal observation.
p38 MAPK Regulates APC and β-Catenin Cellular Localization

Fig. 1. p38 MAPK activity and APC subcellular localization. Primary mouse keratinocytes derived from either wild type (WT) or Gadd45a-null (Gadd45a−/−) mice were transfected with either β-Gal-NLS1 (NLS1) or β-Gal-NLS2 (NLS2) and subjected to immunocytochemistry with anti-β-Gal antibody (red label). In A, irrespective of the construct, β-Gal localization in wt cells was detected at equal frequency in either the nucleus and cytoplasm, while Gadd45a-null cells retained β-Gal predominantly in the cytoplasm. In B, wt cells transfected with β-Gal-NLS1 (NLS1), but not treated with p38 MAPK inhibitor (−Inhib), have either cytoplasmic or nuclear β-Gal localization with similar frequency, while the wt cells treated with the p38 MAPK inhibitor SB202190 (+Inhib) have predominantly perinuclear β-Gal localization (but not nuclear), which is indicative of disruption of nucleocytoplasmic shuttling. Two representative fields for each of the specified culture conditions (−Inhib or +Inhib) are shown. Magnification is ×400.

Fig. 2. p38 MAPK activity and β-catenin subcellular localization. In A, β-catenin (red label) cellular localization was visualized by fluorescent microscopy on cultured primary mouse keratinocytes derived from either wild type (WT) or Gadd45a-null (Gadd45a−/−) mice. While wt cells have β-catenin localize predominantly in the cytoplasm, Gadd45a-null cells have β-catenin restricted to the nucleus. DAPI stain (blue) is used to define the nucleus. In B, wt primary keratinocytes were subjected to SB202190 p38 MAPK inhibitor treatment (+Inhib). While untreated wt cells (−Inhib) have β-catenin localized predominantly in the cytoplasm, β-catenin localization in SB202190-treated cells shifted significantly from the cytoplasmic space to either the nuclear or perinuclear areas. Two representative fields for each of the culture conditions (−Inhib or +Inhib) are shown. Magnification is ×400.

Gadd45a protein leads to three key observations: 1) compromised APC nucleocytoplasmic shuttling, 2) a dramatic increase in cytoplasmic and nuclear β-catenin protein levels, and 3) reduced GSK3β activity as determined by a modest increase in phosphorylated GSK3β, the inactive form of this being kinase. Similarly, wt cells treated with the p38 MAPK inhibitor have altered APC localization accompanied by increased cytoplasmic and nuclear β-catenin.

While untreated wt cells have APC localized predominantly in the nucleus, untreated Gadd45a-null keratinocytes retain APC almost exclusively in the cytoplasm. Moreover, in untreated wt cells, soluble β-catenin levels are relatively low and present exclusively in the cytoplasm. This is in stark contrast to untreated Gadd45a-null cells that have dramatically higher levels of soluble β-catenin protein in both the cytoplasm and nucleus.

Interestingly, while wt cells appear to have more active GSK3β relative to Gadd45a-null cells (as determined by lower levels of phosphorylated protein), SB202190 treatment of wt cells results in only a small change in GSK3β activity (as determined by the level of phosphorylated form) within the 8-h treatment period. This may be attributed to the fact that GSK3β activation is mediated primarily through its direct association with Gadd45a protein (4) and that transient p38 MAPK activity inhibition will not necessarily reduce Gadd45a protein levels and, consequently, not interfere with its ability to regulate GSK3β activity.

Since GSK3β activity does not appear to be substantially compromised within the treatment timeframe, our data indicate that the effects of SB202190 on preventing proper β-catenin degradation are likely due to interference with the nucleocytoplasmic shuttling of APC. As with wt cells, Gadd45a-null keratinocytes demonstrated only a slight decrease in GSK3β activity following SB202190 treatment. This likely results from...
the fact that, as previously established, p38 MAPK and GSK3β activity is already compromised in Gadd45a-null keratinocytes (3, 4).

Thus far, the data presented above strongly support the notion that Gadd45a-dependent p38 MAPK signaling is necessary for proper modulation of soluble β-catenin levels, which involves not only APC complex activation (as defined in Ref. 4) but also involves proper APC cellular localization/trafficking.

p38 MAPK Associates with Both CK2 and PKA and Modulates Their Activity—Due to: 1) our observation that APC is not able to shuttle properly in keratinocytes with inadequate p38 MAPK signaling, along with 2) the recent identification of two APC NLS elements that may be regulated by CK2 and PKA kinases (28, 38), we investigated the possibility that p38 MAPK contributes to APC subcellular localization by directly modulating CK2 and PKA. Immunoprecipitation assays performed with purified GST-p38 revealed that p38 is able to complex with both CK2 and PKA (Fig. 4). To determine the biological significance of this interaction, we performed both CK2 and PKA kinase assays on total cell lysates derived from wt cells (WT), wt cells treated with SB202190 (WT + SB), and from Gadd45a-null cells (Gadd45a−/−). Immunoprecipitates were subject to the respective kinase activity assay as described in the materials and methods section. SB202190 treatment of wt cells results in a 13% increase in PKA activity and a 23% reduction in CK2 activity relative to untreated wt control cells. Similarly, lack of Gadd45a results in a 38% increase in PKA activity and a 53% reduction in CK2 activity relative to untreated wt control cells. Representative results of kinase assays performed in duplicate are shown.

along with the mislocalization of APC in p38 MAPK signaling defective cells, demonstrate the significance of p38 MAPK in enabling APC to properly shuttle in and out of the nucleus and effectively regulate soluble β-catenin levels.

DISCUSSION

The primary function of the APC tumor suppressor is to modulate the expression of β-catenin, which behaves as an oncogene when overexpressed and localized to the nucleus (9, 23, 39). In the nucleus, β-catenin will form an activating transcription factor with TCF and induce proliferation genes such as Cyclin D1 and c-MYC as well as invasion/metastasis genes such as MMP-7, MMP-9 (39). Normally, APC contributes to maintaining relatively low levels of soluble β-catenin by various means: first, in the cytoplasm, APC will form a destruction complex along with PP2A, GSK3β, Axin, CK1ε, and possibly Gadd45a, which targets β-catenin for phosphorylation and ubiquitin/proteasome-mediated degradation. Second, in the nucleus, APC will interfere with β-catenin/TCF complex formation and in essence prevent the activation of genes involved in transformation. Moreover, nuclear APC will promote export of β-catenin back into the cytoplasm where it is subject to degradation or redirected to the cell membrane for assembly at adherens junctions (4, 25, 28, 29, 38, 40).
It is becoming increasingly evident that nucleocytoplasmic shuttling plays a significant role in enabling several tumor suppressors such as APC to effectively regulate cell proliferation, migration, and survival pathways. Not too surprisingly, at least 10 tumor suppressors, including p53, BRCA1, and Smad4, among others, are known to have NLS elements that contribute to their increased nuclear accessibility (25). Recently, two NLSs within the central region of APC and one NLS-independent import element within the N-terminal armadillo-like ARM domain of APC have been identified (28, 31). The NLS elements utilize the importin α/β heterodimeric NLS receptor for entry into the nucleus, while the ARM repeats utilize the B56α regulatory subunit of PP2A to gain entry (25). Although many tumors have truncated APC proteins that lack the central NLS elements, yet retain the potential to shuttle into the nucleus, both NLSs are required for optimal APC nuclear translocation and effective down-regulation of nuclear β-catenin levels (28).

In the same vein, the inability of the APC protein to translocate into the nucleus may contribute to the transformed phenotype of DLD-1 colon cancer cells, for instance, which carry a single, mutant allele coding for a truncated APC protein lacking the NLS elements (32). Therefore, effective translocation of APC into the nucleus is critical for its function as a β-catenin suppressor, and a compromise in its ability to enter the nucleus would have a profound impact on its ability to perform its function as a tumor suppressor.

Previously, we identified Gadd45α as an important component enabling the APC complex to effectively mediate free, cytoplasmic β-catenin levels through: 1) direct association with APC complex proteins and 2) maintaining p38 MAPK activation via a positive feedback loop (3, 4). In this report, we further identify p38 MAPK and Gadd45α as two components of a pathway that promotes efficient APC nuclear translocation and consequently also promotes effective modulation of soluble β-catenin in both the cytoplasm and nucleus. Inadequate p38 MAPK activity, either due to use of inhibitors or due to the absence of Gadd45α (3, 4), prevents APC from translocating into the nucleus and effectively shutting β-catenin out into the cytoplasm for degradation. Consequently, cells lacking Gadd45α and/or adequate p38 MAPK signaling demonstrate increased levels of soluble, oncogenic β-catenin in both the cytoplasmic and nuclear compartments.

Although the end result of increased levels of soluble β-catenin and APC was similar for both Gadd45α-null keratinocytes and p38 inhibitor-treated keratinocytes, the subcellular localization of APC differed somewhat between the groups and appears to be more severe in Gadd45α-null cells. While Gadd45α-null keratinocytes have β-Gal-NLS\textsubscript{APC} localized almost exclusively in the cytoplasm, wt keratinocytes treated with the p38 inhibitor have β-Gal-NLS\textsubscript{APC} aggregate in the perinuclear space. This difference may arise from a combination of factors. First, the effects observed in Gadd45α-null cells correspond to a permanent loss of normal p38 MAPK activation as opposed to a transient interference with p38 inhibitors. As reported previously, Gadd45α not only contributes to maintaining p38 MAPK activity but also to enhancing APC complex function, which likely occurs through its direct interactions with various components of the APC complex (3, 4). Based on our results, loss of normal p38 MAPK signaling will not only impact CK2 activation but also affect PKA suppression, both of which are necessary to effectively mediate APC NLS elements and shuttle APC into the nucleus (Fig. 6). Second, unlike p38 MAPK, Gadd45α may also have a significant impact on APC subcellular localization by possibly contributing to NLS-independent nuclear import. The B56α subunit of PP2A, for instance, is known to associate with the ARM motif of APC and shuttle APC into the nucleus in an NLS-independent manner (31). We have previously reported that Gadd45α directly interacts with PP2A, and this association could conceivably influence and contribute to APC nuclear shuttling (4). Similarly, recent studies demonstrate that Gadd45α may play a role in regulating cyclin B1 activity by not only associating with and disrupting the cdc2-cyclin B1 complex (41) but also by regulating cyclin B1 nuclear shuttling (42).

Therefore, relative to transient p38 MAPK inhibition, permanent loss of Gadd45α may have a more profound effect on APC trafficking due to the additive effect of not only compromising p38 MAPK signaling (which in turn affects CK2/PAK-mediated NLS-dependent trafficking) but also by possibly compromising B56α-mediated APC nuclear import. This may also explain the differences in GSK3β phosphorylation status between the groups. In Gadd45α-null keratinocytes, GSK3β is highly phosphorylated (and presumably inactive) while in wt cells, either control untreated cells or p38 inhibitor-treated cells, the levels of phosphorylated GSK3β were relatively lower, indicative of higher APC complex activity.

In summary, there is increasing evidence that both p38 MAPK and Gadd45α are playing important roles in skin homeostasis and may be acting as tumor suppressors. The effects of p38 MAPK and Gadd45α on APC function involve not only enhancing APC complex activation but, as we describe here, may also involve promoting APC nuclear localization, without which adequate suppression of nuclear β-catenin will not be achieved. We previously established that loss of normal p38 MAPK signaling and/or absence of Gadd45α will result in increased risk for skin tumorigenesis due to loss of normal cell cycle checkpoint activation, diminished sunburn/apoptosis, and reduced inflammatory responses along with possible risk for invasion/metastasis associated with a dramatic increase in MMP expression and activity (3, 4, 43). Our data provide supportive evidence that p38 MAPK and Gadd45α are intimately involved in modulating APC nucleocytoplasmic shuttling, which is critical for its proper functioning as a tumor suppressor targeting soluble β-catenin for degradation and ultimately reducing the risk for malignancy.

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**FIG. 6. Working model.** p38 MAPK directly associates with both CK2 and PKA, which are known to either promote or block APC nuclear translocation, respectively. A, Normal p38 MAPK activity is required to adequately modulate CK2 and PKA, whereby p38 will enhance CK2 activity and suppress PKA activity. Relatively higher CK2 (thick line) activity and lower PKA activity (thin line) will promote APC nuclear translocation (thick line). B, on the other hand, loss of normal p38 MAPK activity due to either lack of Gadd45α protein or use of p38 inhibitors will result in relatively higher PKA activity. This in turn will contribute to a block in APC nuclear translocation.
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