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Id2 Is Dispensable for Myc-Induced Epidermal Neoplasia

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We have previously described a transgenic mouse model of epidermal neoplasia wherein expression of a switchable form of c-Myc, MycERTAM, is targeted to the postmitotic suprabasal keratinocytes of murine epidermis via the involucrin promoter. Sustained activation of c-MycERTAM results in a progressive neoplastic phenotype characterized by aberrant ectopic proliferation and delayed differentiation of suprabasal keratinocytes, culminating in papillomatosis. Transcription of the Id2 gene is regulated by Myc family proteins. Moreover, Id2 is implicated as a pivotal determinant of cell fate in multiple lineages and has a demonstrated role in mediating Myc-dependent cell proliferation in vitro through its interaction with retinoblastoma protein. Using Id2 nullizygous mice, we assessed in vivo the requirement for Id2 in mediating Myc-induced papilloma formation in skin. We show that absence of Id2 has no discernible impact on any measurable attribute of Myc function or on the timing or extent of eventual tumor formation. Thus, our data argue against any essential role for Id2 in mediating Myc action in vivo.

Deregulated expression of myc family oncogenes occurs in a wide range of human cancers and is often associated with aggressive, poorly differentiated tumors (28). As members of the bHLH-Zip transcription factor family, Myc proteins are thought to exert their biological effects by modulating, either positively or negatively, expression of downstream target genes (24). Indeed, recent advances in expression array and serial analysis of gene expression technologies have indicated that Myc proteins regulate a bewilderingly diverse variety of genes which are variously implicated in almost every aspect of cellular activity, including proliferation and differentiation, growth and apoptosis, metabolism, intracellular transport and organization, and interactions with the somatic cell environment (6, 10, 27, 31, 41). The breadth of potential Myc target genes, together with the highly contingent nature of the pathways and processes they oversee, makes it a great challenge to determine which attributes of Myc proteins are most critical for their tumorigenic properties.

The Id2 gene was recently identified as a direct transcriptional target of both N-Myc and c-Myc (23). Using a conditional form of N-Myc (N-MycER), in which N-Myc activity is rendered dependent on the presence of the ligand 4-hydroxytamoxifen (4-OHT) by fusion with the estrogen receptor ligand binding domain (25), activation of N-Myc was demonstrated to rapidly induce expression of Id2 at both mRNA and protein levels. Induction of Id2 by N-Myc (and by c-Myc) is dependent on the presence of two Myc consensus E boxes located within the Id2 5′ regulatory region. Consistent with this, chromatin immunoprecipitation analysis has indicated binding of c-Myc/Max heterodimers to the Id2 promoter (23). Moreover, transforming growth factor β-mediated inhibition of Id2 expression is associated with displacement of Myc/Max heterodimers from, and enhanced binding of Mad4/Max heterodimers to, the Id2 promoter and correlates with proliferative arrest in both mouse mammary epithelial cells and human keratinocytes (42). Intriguingly, the combination of deregulated Myc and constitutively activated Ras fails to transform Id2-deficient mouse embryonic fibroblasts in vitro (23; D. J. Murphy and M. A. Israel, unpublished results), intimating that Id2 may mediate a Myc-dependent function(s) necessary for cell transformation. An additional study revealed a close correlation between N-Myc amplification and Id2 expression in neuroblastomas, implicating Id2 as a potential prognostic indicator for this disease (21), although more recent analyses have questioned this particular finding (40, 44, 45).

Id2 is one of four related and evolutionarily conserved helix-loop-helix (HLH) proteins that dimerize with the various basic region HLH (bHLH) transcription factors whose members modulate diverse aspects of cell differentiation, proliferation, and survival (29, 30, 48). Because the Id proteins lack a DNA binding basic motif, their heterodimerization with bHLH proteins prevents the latter from binding E-box DNA elements. Consequently, Id proteins act as endogenous negative regulators of bHLH function (3, 17). Indeed, Id proteins are widely expressed during embryogenesis and appear to be intimately involved in regulating cellular differentiation in a spectrum of lineages (15, 16, 37).

Several in vitro studies have indicated that Id proteins promote cell proliferation. Thus, antisense blockade of Id1, Id2, and Id3 expression significantly extends the G1 phase of the cell cycle (1, 11), while both Id2 and Id4 bind the retinoblastoma gene (Rb) product Rb and related proteins, p107 and p130, antagonizing their ability to repress E2F-dependent transcription and cell cycle progression (13, 22; previously discussed in reference 29). Moreover, Id1 (and probably other Id proteins) blocks the ability of the ubiquitous bHLH protein E2A to induce expression of the cell cycle inhibitor p21 (35), while Id4 blocks E protein-mediated induction of p21(16,14), p15(16,11), and p16(16,14) promoter activity (33). In addition, Id1, Id2, and Id3 can all inhibit Ets domain transcription factors

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animal research at the University of California, San Francisco. Inv-MycERTAM mice, on a mixed C57Bl6/CBA background, were bred to Id2 heterozygous mice, on a 129 background, and backcrossed to yield Inv-MycERTAM/Id2-null and Inv-MycERTAM/Id2-wt offspring. Genotyping was performed on tail tip DNA by PCR. The following primers were used: MycG (forward, AGGCGGCACTGTTCAAGGTCA) and MycERTAM (reverse, CCAAGAGTTTCGACCGCTCTAGTGTCA). Id2-105 (forward, AGGGCCCACTGTCGTCCCTGTAAC); Id2-3 (wt) (reverse, CAAACCTGATGCCCTTCTGAG); and Neo-8 (Id2 null) (reverse, TAGCGTGGAAAGCGATCAGCG).

Two- to four-month-old mice were shaved to reveal an ~2 cm2 area of dorsal skin and topically treated daily with 200 nl of 4-OHT (5 mg/ml) in ethanol or 200 nl of ethanol alone. Two to three mice of each genotype were euthanized with CO2, followed by cervical dislocation, at each indicated time point. Skin tissue was harvested from the treatment area, bisected, either frozen fresh in OCT (TissueTek) or fixed in 4% neutral buffered paraformaldehyde (PFA), dehydrated, and embedded in paraffin.

In situ hybridization. Antisense RNA probes were transcribed in vitro from linearized plasmid template DNA in the presence of 35S-UTP. For probe to Id2 expression, a 235-bp XbaI-Sall fragment representing 3’ untranslated sequence, subcloned into pBluescript SK (Stratagene), was linearized with BglII and transcribed with T7 RNA polymerase. To probe for Id3 expression, a 443-bp EcoRI fragment representing 3’ untranslated sequence, subcloned into pCR1 (Invitrogen), was linearized with NotI or XhoI and transcribed with SP6 RNA polymerase. ODC-1 probe was generated against a ~530-bp HindIII-NcoI fragment from the 3’ end of the ODC-1 cDNA, subcloned into pBluescript SK, linearized with HindIII, and transcribed with T7 RNA polymerase. Probes were hydrolyzed to ~300 bp (Id2 probe excepted) in 2X sodium carbonate buffer (1 M NaHCO3, 1 M Na2CO3, pH 10), neutralized with acetic acid (to a final concentration of 0.3%), precipitated in ethanol, and resuspended in 10 mM dithiothreitol.

Ten-micrometer-thick frozen tissue sections were thawed, air dried, and pre-treated at room temperature (RT) for hybridization as follows: 20 min in 4% PFA, 4 min in 3X phosphate-buffered saline (PBS), twice for 4 min in 1X PBS, air dried briefly, 20 min in proteinsize K solution (100 mM Tris [pH 8.0], 50 mM EDTA-1 µg of proteinsize K/ml), 5 min in 4% PFA, 4 min in 3X PBS, twice for 4 min in 1X PBS, 15 s in diethyl pyrocarbonate-treated distilled water, air dried briefly, 10 min in 200 ml of 0.1 M triethanolamine containing 500 µl of acetic anhydride, rinsed twice in diethyl pyrocarbonate-treated distilled water, and air dried prior to hybridization.

Riboprobes (103 dpi/slide) were heat denatured at 85°C for 5 min and mixed with hybridization solution (50% [vol/vol] formamide, 10% [vol/vol] dextran sulfate, 2X Denhardt’s solution, 5X SSC [1X SSC is 0.15 M NaCl plus 0.015 M sodium citrate]), 10 mM β-mercaptoethanol, 250 µg of yeast RNA/ml, 500 µg of single-stranded DNA/ml (~ 100 µl/slide) preheated to 55°C. Probe was added directly to sample slides, which were then covered with Parafilm and incubated overnight at 55°C in a hummid chamber. After hybridization, Parafilm strips were removed and slides were washed in the following solutions before being dehydrated through graded ethanol and air dried: 30 min at 55°C in 50% formamide-2X SSC-1 mM EDTA-10 µM β-mercaptoethanol, 30 min at RT in 2X SSC, 1 h at RT in 2X SSC-1 mM EDTA-20 µg of RNase A/ml-1 U of RNase T1/ml, two times for 30 min at 55°C in 2X SSC, and 5 min at RT in 0.2X SSC. Radiolabeled slides were dipped in photographic emulsion (Kodak) mixed at a ratio of 1:1 with 1% glycerol, dried, and stored for 4 to 10 days. Slides were then immersed in developing solution (Kodak no. 146-4933) for 5 min at 16°C, rinsed in water, and fixed (Kodak fixer no. 197 1746) and then dehydrated through graded ethanol and mounted with coverslips. Labeled sections were analyzed by dark-field imaging using a Leica DM RXA microscope.

Immunohistochemistry. The primary antibodies used were anti-Ki-10 (1:2,000 dilution) and anti-K14 (1:10,000 dilution; Covance PRB-155p and PRB-159p), anti-PCNA (1:100 dilution; Biogenes-mx200-uu), CD-31 (1:50 dilution; Pharmingen 01951D), and anti-Id1 (1:50 dilution) and anti-Id3 (1:50 dilution; Santa Cruz sc488 and sc490). Immunohistochemistry and immunofluorescence analyses were performed on 5-µm thick paraffin-embedded sections as previously described (34), with the exception of CD31 staining, which was performed on 10-µm-thick frozen sections. Briefly, paraffin-embedded slides were deparaffinized and rehydrated through graded ethanol. For MCM-7, PCNA, and Ki14 antigen retrieval was performed by microwaving on low power for 5 min in 10 mM sodium citrate (pH 6.0). Sections were incubated in blocking solution (4% bovine serum albumin–3% preimmune goat serum–2% fish gelatin) for 1 h at RT, and primary antibodies were added in blocking buffer and incubated over-night at 4°C. Secondary antibodies were diluted 1:200 in blocking solution and incubated at RT for 1 h. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit (Pierce) was used to identify Ki10 and K14 staining. HRP-conjugated goat anti-mouse immunoglobulin M (Vector) was used for PCNA staining, Alexa
Id2 expression was assayed by in situ hybridization, while adjacent sections were probed for expression of the well-characterized Myc target gene ODC-1 to confirm Myc transcriptional activity (Fig. 1). As a negative control, we used antisense riboprobe for Id4, which is not expressed in skin (16). ODC-1 expression was detectable in all nucleated keratinocytes, with essentially uniform expression throughout the epidermis, although in this case a gradient of expression was apparent, with highest expression of Id2 in the inner (i.e., basal and immediately suprabasal) layers. This gradient in expression is consonant with that of the proliferation markers PCNA and Ki67 (34; I. Flores, D. J. Murphy, L. Brown Swigart, and L. Evan, unpublished data). Thus, the pattern of Id2 expression correlates with the proliferative state of keratinocytes rather better than with Myc activity, as assessed by ODC-1 expression. Id4 expression was undetectable anywhere within either normal or hyperplastic epidermis.

Id2 is dispensable for development and full progression of Myc-induced papillomas. To determine the extent to which Id2 expression might be required for any aspect of the Myc-induced papilloma phenotype, Inv-MycERTAM mice were crossed with Id2-deficient mice and then backcrossed to yield either Inv-MycERTAM/Id2-null or Inv-MycERTAM/Id2-wt offspring. Two- to four-month-old mice of appropriate genotypes were then treated topically with 4-OHT as before, and littermate transgene-negative mice were treated similarly to serve as controls. At the end of the 2-week treatment, Inv-MycERTAM skin was visibly thickened and flaking, as described previously (34). Mice were then euthanized, and the treated area of skin was harvested. Half of the sample was fixed with 4% PFA and paraffin-embedded, while the other half was snap frozen.

Histological analysis of Inv-MycERTAM skin revealed the epidermis of 4-OHT-treated mice to be several layers thicker than that of transgene-negative control samples. Immunohistochemistry confirmed the marked accumulation of less differentiated suprabasal keratinocytes. Expression of Id2 was then assayed by in situ hybridization, while adjacent sections were probed for expression of the well-characterized Myc target gene ODC-1 to confirm Myc transcriptional activity (Fig. 1). As a negative control, we used antisense riboprobe for Id4, which is not expressed in skin (16). ODC-1 expression was detectable in all nucleated keratinocytes, with essentially uniform expression throughout the epidermal strata. Id2 was also detected throughout the epidermis, although in this case a gradient of expression was apparent, with highest expression of Id2 in the inner (i.e., basal and immediately suprabasal) layers. This gradient in expression is consonant with that of the proliferation markers PCNA and Ki67 (34; I. Flores, D. J. Murphy, L. Brown Swigart, and L. Evan, unpublished data). Thus, the pattern of Id2 expression correlates with the proliferative state of keratinocytes rather better than with Myc activity, as assessed by ODC-1 expression. Id4 expression was undetectable anywhere within either normal or hyperplastic epidermis.

Id2 is not required for Myc-induced keratinocyte proliferation. Although absence of Id2 had no overall effect on Myc-induced papillomatosis, it remained possible that lack of Id2 might compromise specific aspects of Myc function that are then compensated for in some way. For this reason, we assessed separately the effects of Id2 loss on Myc-induced keratinocyte proliferation and apoptosis.

In normal epidermis, proliferating keratinocytes are present only within the basal layer: as cells detach from the basal lamina and migrate into the suprabasal compartment, they drop out of cycle and differentiate (8). In contrast, Myc-induced epidermal hyperplasia in Inv-MycERTAM mice is characterized by the presence of proliferating keratinocytes several layers into the expanded suprabasal compartment. To assess whether the absence of Id2 affected Myc-induced keratinocyte proliferation, we used antibodies specific for the cell proliferation marker PCNA to analyze immunohistochemically the proliferative status of skin cells (Fig. 3). As expected, activation of Myc in the suprabasal compartment of murine epidermis

488-conjugated goat anti-rabbit was used to detect anti-Id1 and Id3 antibodies, and Alexa 488-conjugated goat anti-rat (Molecular Probes) was used to detect CD-31. Alexa 488-stained sections were counterstained with propidium iodide (1 μg/ml) in fluorescent mounting medium (Dako) and analyzed by fluorescence microscopy. HRP-labeled sections were incubated with ABC reagent (Vector) and developed with diaminobenzidine (DAB). DAB-stained slides were counterstained lightly with hematoxylin, dehydrated through graded ethanols, mounted with coverslips, and analyzed by light microscopy.
resulted in the progressive accumulation of cycling suprabasal keratinocytes in Inv-MycERTAM/Id2-wt mice. Within 7 days of onset of sustained MycER\textsuperscript{TAM} activation, PCNA expression was clearly evident in the immediately suprabasal cell layers. By 3 weeks, PCNA expression extended all the way through the hyperplastic suprabasal skin to the granular layer. Essentially identical staining was observed using antibodies specific for the DNA replication licensing factor MCM-7 (data not shown). We could discern no difference in the onset or prevalence of Myc-induced suprabasal keratinocyte proliferation in the absence of Id2 nor on the polarity of accumulation (from the basal layer outwards).

Negligible apoptosis accompanies Myc-induced epidermal hyperplasia in Id2-wt mice. By 3 weeks, PCNA expression extended all the way through the hyperplastic suprabasal skin to the granular layer. Essentially identical staining was observed using antibodies specific for the DNA replication licensing factor MCM-7 (data not shown). We could discern no difference in the onset or prevalence of Myc-induced suprabasal keratinocyte proliferation in the absence of Id2 nor on the polarity of accumulation (from the basal layer outwards).

Negligible apoptosis accompanies Myc-induced epidermal hyperplasia in Id2-wt mice (34; Flores et al., unpublished). We observed a similar absence of detectable keratinocyte apoptosis in the absence of Id2 (data not shown).

Id2 expression is not required for c-Myc to delay keratinocyte differentiation. The augmented proliferation and hyperplasia of suprabasal keratinocytes induced by activation of MycER\textsuperscript{TAM} is accompanied by accumulation of less differentiated suprabasal cells. This is manifested by the persistent expression of basal markers such as keratin K14 and p63 out into the suprabasal layers together with a delayed or reduced expression of suprabasal cell markers such as involucrin and keratins K1 and K10 (34; Flores et al., unpublished). To explore any influence of Id2 absence on this aspect of Myc action, we examined the effects of Myc activation in Id2-wt and Id2-null mice immunohistochemically by using basal and suprabasal epithelial markers.

In normal epidermis, K10 is expressed exclusively in the spinous and granular layers (5). After 3 weeks of sustained suprabasal MycER\textsuperscript{TAM} activation, expression of K10 becomes progressively lost from the inner suprabasal layers, shifting outwards into the greatly thickened spinous and granular layers (Fig. 4A). In contrast, expression of the basal marker K14 progressively extends out from the basal compartment into the suprabasal one in a manner reciprocal to that of K10 (Fig. 4B). Such apposition was identical in Id2-null mice. The absence of Id2 altered neither the timing nor the extent of these Myc-
induced effects, challenging the notion that Id2 is a key mediator of Myc’s ability to disrupt differentiation. Id2 is dispensable for Myc-induced angiogenesis. Although double knockout Id1/Id3 mice die in utero, Id1-heterozygous/Id3-null and Id1-null/Id3-heterozygous mice are viable and, moreover, exhibit profound resistance to tumor xenografts because of a defect in tumor angiogenesis (26). Id1, Id2, and Id3 are all expressed in the endothelial cells lining the vasculature, with the exception that Id2 is absent from central nervous system vasculature (2, 4). Although Id2-deficient mice are viable, with no apparent deficit in vasculature, there remained the possibility that Id2 status might affect the profound angiogenesis that suprabasal activation of c-Myc elicits in adjacent dermis, most notably in areas of papilloma formation (34). We used the marker of vascular endothelium, CD31, to explore any role for Id2 in Myc-induced skin angiogenesis (Fig. 5). In both Inv-MycER$^{TAM}$/Id2-null and Inv-MycER$^{TAM}$/Id2-wt mice, activation of Myc for 2 to 3 weeks induced similar robust dermal angiogenesis in the region of skin treated with 4-OHT. Thus, Id2 appears not to be required for Myc-dependent angiogenesis. Id1 and Id3 are abundantly expressed in hyperplastic Inv-MycER$^{TAM}$ epidermis. Although there is no evidence that any other member of the Id family is regulated by Myc, it is possible that the lack of a discernible influence of the absence of Id2 upon the Myc-induced papillomatous phenotype in Id2-null animals is due to functional redundancy with other expressed Id proteins. We therefore investigated whether other Id proteins are expressed in the hyperplastic epidermis of 4-OHT-treated Inv-MycER$^{TAM}$ skin.

Myc was activated for 14 days in Inv-MycER$^{TAM}$ mice, either Id2-wt or Id2-null, and sections were then assayed for expression of Id1 and Id3 by immunofluorescence. Abundant cytoplasmic Id1 staining was observed in all nucleated layers from hyperplastic epidermis of both Id2-wt and Id2-null mice. Id3 was also expressed in all nucleated keratinocytes in both Id2-wt and Id2-null mice, although in contrast to what was seen with Id1, Id3 staining was predominantly nuclear (Fig. 6). These
results concur perfectly with those reported previously for expression of Id proteins in samples of human squamous cell carcinoma of the skin (19). The presence or absence of Id2 had no significant effect on expression of either Id1 or Id3. Thus, both Id1 and Id3 are expressed in Myc-induced hyperplastic epidermis. However, neither Id1 nor Id3 expression correlates with Myc-induced keratinocyte proliferation nor indeed with the differentiation status of Inv-MycERTAM suprabasal keratinocytes. It is therefore unlikely that either Id1 or Id3 mediates Myc’s tumorigenic function in this tissue.

**DISCUSSION**

We used a switchable MycER<sup>TAM</sup> transgenic approach to investigate the potential role for Id2 in Myc-dependent tumorigenesis in skin in vivo. Sustained activation of MycER<sup>TAM</sup> in the suprabasal keratinocytes of murine epidermis results in a complex neoplastic phenotype, characterized by progressive hyperplasia accompanied by anaplasia, dysplasia, and angiogenesis and culminating in papillomatosis. Despite the published evidence indicating that Id2 is a pivotal proliferative effector of Myc (21, 23) and the abundance of Id2 transcripts in the MycER<sup>TAM</sup>-induced skin hyperplasia, we found that deletion of the Id2 gene has no detectable influence on the extent or the kinetics of the complex neoplastic phenotype elicited by Myc in skin.

Substantial data exist to implicate Myc family proteins in the regulation of Id2 transcription. The Id2 promoter contains multiple E-box sequences which, by transient reporter analysis, are required both for induction by c-Myc and N-Myc and also for transforming growth factor β-dependent repression of Id2, demonstrably mediated in part by the Myc antagonist Mad-4. Direct binding of c-Myc/Max and Mad-4/Max complexes to the Id2 promoter in vivo has also been demonstrated in both fi-
broblasts and immortalized keratinocytes by chromatin immuno-
precipitation (23, 42). Moreover, while the initial report
suggesting a close correlation between N-Myc amplification
and Id2 expression in neuroblastoma (21) has recently been
called into question (40, 44, 45), some correlation be-
centre: Id-Myc and Id2 expressions appears to exist although Id2 still
lacked prognostic value for neuroblastoma in this and other
studies (40, 45). However, regulation of the Id2 promoter
is quite complex. Id2 promoter activity is induced by a number of
mitogenic signals and fluctuates in response to various tissue
culture conditions, such as plating and refeeding of cells (30,
48). In addition to the potential Myc-binding E boxes, the Id2
promoter also contains at least one SP-1 site and is, fur-
thermore, bound by Ets domain and ATF family transcription
factors (9, 18) and activated by both BMP4 and STAT3 (12,
36). Given that the bulk of the present evidence suggests only
a modest (i.e., approximately twofold) influence of Myc on Id2
expression, it seems unlikely that Myc is the decisive arbiter of
Id2 transcription. Consistent with this, our own in situ analysis
suggests that while Myc may indeed contribute to Id2 tran-
scription in hyperplastic keratinocytes, the principal determi-
nant of Id2 expression is the overall proliferative status of
keratinocytes in which it is expressed.

Generally speaking, Id proteins are highly expressed in rap-
idity dividing and less differentiated cell populations. We found
that Id1, Id2, and Id3 are all expressed in the hyperplastic
epithelium of 4-OHT-treated Inv-MycERTAM skin, in close
agreement with the results of a previous report demonstrating
Id protein expression in human squamous cell carcinoma of
the skin (19). It is possible that the extensive expression of Id1
and Id3 functionally compensates for loss of Id2 in the Myc-
induced phenotype. All four Id proteins can bind the ubiqui-
tously expressed E-box proteins E2A, HEB, and E2-2, disrupt-
ing their interaction with tissue-specific bHLH transcription
factors (30). Expression of E2A has been demonstrated in
keratinocytes, although tissue-specific interacting proteins that
regulate epidermal lineage commitment have yet to be defined
(19). Furthermore, Id proteins can bind tissue-specific bHLH
proteins (e.g., MyoD, Neuro D, and Mash 1) directly, although
they do so with differing affinities (21). Id1, Id2, and Id3 are
each also capable of binding paired domain transcription fac-
tors, Pax-2, Pax-5, and Pax-8, as well as Ets domain proteins
Etk-1 and Sap-1, although, again, they each exhibit varying
affinities for these factors (38, 47). Thus, there is ample poten-
tial for degeneracy of function between Id family proteins that
might compensate for loss of any one during development.
Nonetheless, whereas Id2 mRNA expression correlates well
with the delay in differentiation of MycERTAM keratinocytes,
no such correlation is evident for Id1 or Id3. This absence of
any such correlation between Id1 or Id3 expression and either
keratinocyte proliferation or the Myc-induced disruption of
keratinocyte differentiation strongly argues against any role for
Id1 or Id3 in mediating Myc’s tumorigenic properties in this
tissue, either in the presence or absence of Id2.

By crossing our Inv-MycERTAM mice into an Id2-null back-
ground, we were able to interrogate directly the potential role
of Id2 as a mediator of Myc’s oncogenic properties in vivo.
Careful analysis of MycERTAM-induced epidermal neoplasia
throughout its progression revealed no obvious phenotypic
deficit ascribable to the absence of Id2 expression. The rate
and extent of keratinocyte proliferation, delay in keratinocyte
differentiation, dermal angiogenesis, and eventual papiloma-
tosis elicited by Myc were all identical in the presence or
absence of Id2. While it remains possible that Id2 may still
mediate critical Myc functions in specific tissues or tumor
types, our studies indicate that Id2 is not a universal require-
ment for any of the diverse aspects of Myc’s biological func-
tion.

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REFERENCES

Sci. USA 91:4985–4988.


1990. The protein Id: a negative regulator of helix-loop-helix DNA binding

Oncogene 20:8334–8341.

Tyring. 1995. Gene expression of markers associated with proliferation and
differentiation in human keratinocytes cultured from epidermis and from

microarrays reveals that MYC regulates genes involved in growth, cell cycle,

7. de Bruin, A., L. Wu, H. I. Saavedra, P. Wilson, Y. Yang, T. J. Rosol, M.
Weinstein, M. L. Robinson, and G. Leone. 2003. Rb function in extraembry-
onic lineages suppresses apoptosis in the CNS of Rb-deficient mice. Proc.
Natl. Acad. Sci. USA 100:6546–6551.


an oncogenic helix-loop-helix protein, is mediated by the chimeric EWS/Ets


Oda. 1994. Id-related genes encoding helix-loop-helix proteins are required
for G1 progression and are repressed in senescent human fibroblasts. J.

Id genes are direct targets of bone morphogenetic protein induction in

helix-loop-helix protein Id-2 enhances cell proliferation and binds to the

tani, J. H. Carter, and C. M. Julin. 1999. Id gene expression as a key

family exhibits a unique expression pattern in mouse gastrulation and neu-

16. Jen, Y., K. Manova, and R. Benezra. 1996. Expression patterns of Id1, Id2,
and Id3 are highly related but distinct from that of Id4 during mouse em-

17. Kadesch, T. 1993. Consequences of heteromeric interactions among helix-

function of the myogenic helix-loop-helix transcription factor MyoD.

expressed in mouse epidermis and dysregulated in squamous cell carcinoma.
The Cancer Res. 60:5929–5933.


