human nodular BCCs arise from the hair follicle bulge (Jih et al., 1999).

Other types of mouse models for basal cell carcinoma depend on overexpression of genes in the hedgehog pathway, such as Gli and Smo; for example, targeting of Gli expression to the follicle and IFE results in the formation of basal cell tumors that clinically resemble human basal cell carcinomas in that they have a translucent appearance and the presence of small vessels known as telangiectasias (Grachtchouk et al., 2000; Nilsson et al., 2000). These tumors are dependent on continuous Gli expression and regress if the transgene is turned off. Results of early clinical trials suggest that human BCCs are similarly "addicted" to hedgehog signaling and may be amenable to targeted therapy.

Dlugosz and colleagues previously published that constitutive overexpression of activated Smo in the epidermis resulted in basaloid hamartomas (Grachtchouk et al., 2003). These investigators were careful to distinguish between basaloid hamartomas and BCC because basaloid hamartomas, both in humans and in mice, have limited growth potential and rarely develop into BCC. In a more recent study, Blanpain and colleagues also overexpressed activated Smo in the epidermis, but with an inducible system, and described the formation of "basal cell carcinomas." (Youssef et al., 2010) One problem with both the Blanpain and the Wang paper rests on whether the tumors that developed are truly BCCs or whether they are basaloid hamartomas. Input from a dermatopathologist is essential for making the distinction, but marker studies would be ideal. Nonetheless, these findings do suggest that non-bulge cells have a lower threshold than bulge cells for tumor development in response to oncogenic Smo.

Wang et al. suggest that loss of p53 triggers Smo expression in epidermis of *Ptch1*$^{+/-}$ mice. Since Smo is an obligatory activator of Hh signaling, the resultant epidermal BCCs in irradiated p53-deficient *Ptch1*$^{+/-}$ mice suggests that loss of p53 may be a primary event in BCC formation, operating through the novel mechanism of Smo upregulation. This important concept deserves testing in both human epidermis with known p53 mutations and in mouse models. The findings could impact on future targeting of incipient BCC with chemotherapeutic agents.

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Metastatic Colon Cancer Cells Negotiate the Intravasation Notch

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In this issue of *Cancer Cell*, Sonoshita et al. report that Aes/Grg5 prevents metastasis of colorectal cancer cells by sequestering and inactivating Notch transcriptional effectors in distinct nuclear foci. Loss of Aes/Grg5 in invasive cancer cells where Notch is activated by stroma-expressed ligands promotes invasion, transendothelial migration, intravasation, and metastasis.

Metastatic disease is the major cause of cancer-associated death. During the metastatic process, cancer cells need to overcome a number of hurdles, including invasion into neighboring tissue, intravasation into blood or lymphatic vessels, survival in the circulation, extravasation from vessels at distant organs, and colonization and outgrowth at the distant sites. Each of these events involves a number of signaling pathways. In this issue of *Cancer Cell*, Sonoshita and

coworkers (Sonoshita et al., 2011) report that the transcriptional regulator Amino-terminal Enhancer of Split (Aes) or its mouse homolog Groucho gene-related protein 5 (Grg5) represses specific steps of the metastatic spread of colorectal cancer cells by inhibiting Notch signaling.

Sonoshita et al. first identified Aes/Grg5 as a highly expressed gene in primary tumors of Colon26 cancer cells transplanted into syngeneic Balb/c mice, yet absent in liver and lung metastasis derived thereof. Aes/Grg5 expression is also found to be low in liver metastasis of colorectal cancer patients. Notably, cells at the invading front of transplanted cancer cells as well as in human primary colorectal cancers also lack Aes/Grg5 expression, and the loss significantly correlates with tumor invasion and progression stage. Indeed, genetic and pharmacological gain and loss of function experiments with transplantation models of colon cancer demonstrate that Aes/Grg5 efficiently suppresses cell invasion and metastasis without affecting primary tumor growth.

Aes/Grg5 is the smallest member of a family of conserved non-DNA binding transcriptional regulators, the Transducin-like Enhancer of Split (TLE) proteins and their mouse homologs, the Grg transcriptional regulators (Beagle and Johnson, 2010). In contrast to the exclusive transcriptional repression exerted by the long TLEs/Grgs, Aes/Grg5 acts as both repressor and activator of transcription and is critical in a variety of biological processes (Beagle and Johnson, 2010). The TLE/Grg proteins have also been implicated in the regulation of pathways well known to affect tumor progression, including the Wnt, TGFβ, Hedgehog, and Notch signaling pathways. Indeed, Sonoshita et al. find an efficient, dose-dependent repression of Notch signaling by Aes/Grg5, while it only marginally stifles Wnt signaling and has no effect on TGFβ or Hedgehog signaling. In contrast, TLE1, another member of the TLE/Grg family, fails to repress Notch signaling by itself, yet it physically interacts with and potentiates the inhibitory activities of Aes/Grg5.

The authors go on to delineate the mechanism by which Aes/Grg5 represses Notch-mediated signal transduction. In mammals, Notch signaling is activated by binding of transmembrane ligands, such as Delta-like (Dll1-4), Serrate and Jagged1-2, to the transmembrane Notch receptors (Notch 1-4) (Kopan and Ilagan, 2009). Ligand binding promotes cleavage of the Notch receptors and the release of the Notch intracellular domain (NICD). NICD then translocates to the nucleus and associates with the DNA-binding protein CSL (Rbpjκ in mouse) and its coactivators Mastermind-like (Maml1-4) or the nuclear corepressor SMRT (Silencing Mediator for Retinoid and Thyroid receptors) (Kopan and Ilagan, 2009). Sonoshita et al. show that ectopically expressed GFP-Aes localizes diffusely in the cytoplasm and the nucleoplasm. Rbpjκ, NICD, and Maml1 also showed diffuse nucleoplasmic localization in these cells. Coexpressing TLE1, however, resulted in localization of GFP-Aes to distinct nuclear foci, which also contain Rbpjκ, NICD, and Maml1. Lack of transcription in these foci is reminiscent of the Bach2 foci or the matrix-associated deacetylase (MAD) bodies containing histone deacetylases (HDAC), the Notch signaling corepressor SMRT, and a number of other transcriptional regulators and chromatin modifiers (Hoshino et al., 2007; Downes et al., 2000). These data suggest that Aes/Grg5 represses Notch signaling by sequestration and inactivation of the Notch-activated transcription complex in distinct foci of the nuclear matrix (Figure 1). Whether Aes/Grg5 is actively contributing to HDAC-mediated transcriptional repression warrants further investigation.

Sonoshita et al. also address the mechanism underlying Notch signaling activation in cancer cells to promote metastasis formation. In their Colon26 transplantation model, Notch receptors are expressed on cancer cells, whereas the ligands are found on stromal cells (Jagged1 in endothelial cells of tumor-associated blood vessels and Dll4 on endothelial cells, smooth muscle cells, and macrophages). Notch signaling activation is mainly found in cancer cells in the vicinity of blood vessels in primary tumors. In metastatic lesions, Notch-activated cells are either found in micrometastasis or in the outer rim of larger metastasis next to stromal cells. Hence, Notch signaling is high in cancer cells adjoining blood vessel and stroma, where Aes/Grg5 is found low and where a high number of cancer cells have entered into blood vessels (intravasation; Figure 1). In vitro transendothelial migration (TEM) assays with endothelial cells expressing Notch ligands and cancer cells expressing Notch receptors support the notion that Notch activation by a tumor-stroma interaction is critical for cancer cell intravasation. Knockdown of Aes/Grg5 in the cancer cells results in increased TEM, whereas ablation of Rbpjκ represses TEM. These data indicate that Notch ligands on endothelial and stromal cells induce Notch signaling in cancer cells and thus promote their TEM.

Finally, the authors have generated mice carrying conditional alleles of Aes/Grg5 to demonstrate that the lack of Aes/Grg5 in the intestinal epithelial tumor cells of $Apc^{\Delta 716}$ mice leads to increased tumor invasion, abundant intravasated cells, and tumor embolism. The marked increase in tumor invasion is dependent on Notch signaling, since it is repressed by pharmacological inhibition. Interestingly, despite increased invasion, no signs of epithelial-mesenchymal transition (EMT) and no metastasis have been observed in these mice.

Altogether, these data show that Aes/Grg5 is a metastasis suppressor gene, mainly by preventing Notch signaling and Notch-mediated local invasion and intravasation. Thus, Notch appears to contribute to the early stages of the metastatic process but does not affect primary tumor growth or metastatic outgrowth per se (Sonoshita et al., 2011). On the other hand, Notch is known to modulate cell apoptosis, survival, differentiation, and EMT. The reason for the conflicting observations may lie in the variety of cancer types studied or in the genetic context of the cancer cells used in the studies. Notably, Colon26 cells carry a Ras mutation, and upon transplantation they show invasion, intravasation, and form metastasis. Composite $Apc^{\Delta 716}$/Aes knockout mice do not carry a Ras mutation and exhibit intravasation yet show no malignant tumor progression and metastasis. In contrast, a mouse model carrying both an APC and a Ras mutation [KRAS(V12G)/Apc(+/1638N)] resembles colorectal cancer in humans, with tumor invasion, intravasation, and metastasis (Janssen et al., 2006). Finally, *cis*-$Apc^{\Delta 716}$/Smad4 mice show local invasion, yet no intravasation, with a significant infiltration of immature myeloid cells

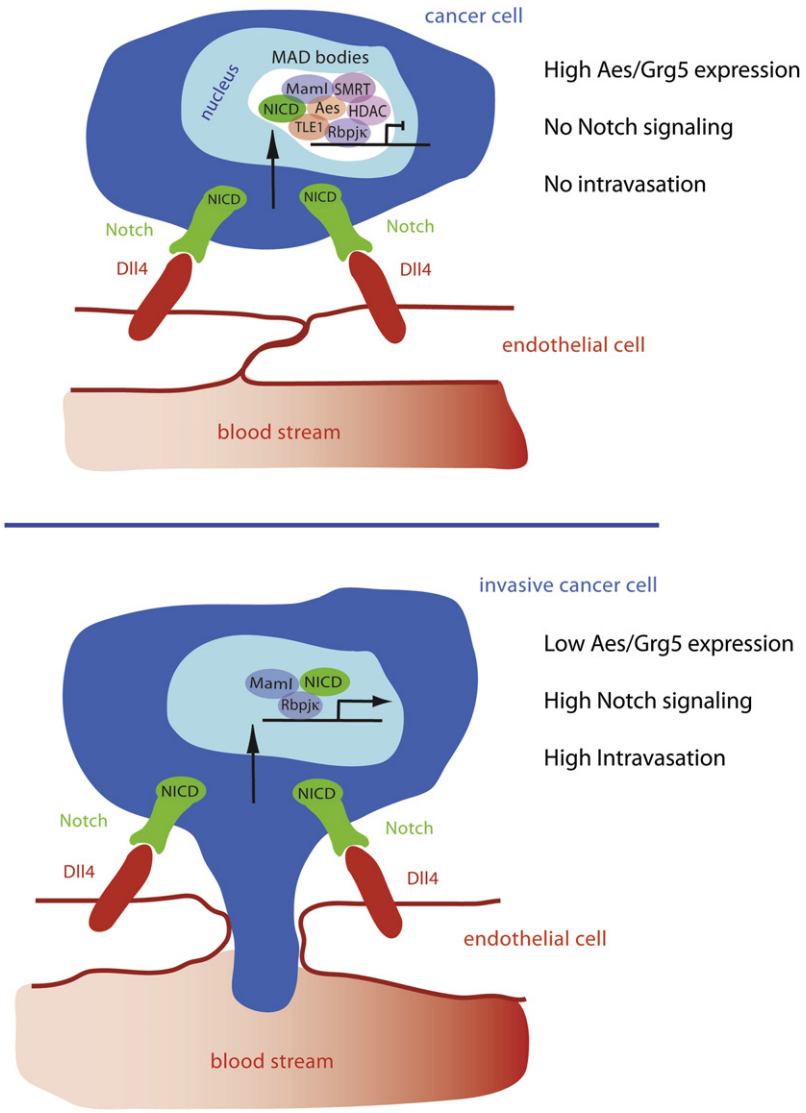

Figure 1. Model of the Mechanisms by which Aes/Grg5 Modulates Notch Signaling, Cancer Cell Intravasation, and Metastasis
For details, see text.

required for tumor invasion (Kitamura et al., 2007). Thus, the role of Notch may differ between the various settings, and other signaling pathways certainly contribute as well. On the other hand, Aes/Grg5 expression inversely correlates with tumor progression in various cancer types (Sonoshita et al., 2011), suggesting that the metastasis suppressor may be active in many cancer types.

It remains open how the expression of Aes/Grg5 is repressed in the invasive cancer cells that are exposed to Notch signaling activation. A preliminary analysis by Sonoshita et al. has not revealed any changes in DNA methylation, and no mutations in the Aes/Grg5 genes have been found so far. Thus, for this type of invasion program, notably in the absence of EMT, Notch receptor activation by ligands on neighboring stromal cells and the concomitant loss of Aes/Grg5 expression in cancer cells is critical. Conversely, the lack of Notch signaling in cancer cells, for example by the lack of access to Notch ligands on stromal cells or by the presence of Aes/Grg5, may reverse the invasion program, a notion that may set the stage for the design of novel anti-metastatic therapy.

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