Are oncogenes sufficient to cause human cancer?

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A fundamental aim of cancer research is to identify the molecular changes that cause normal cells to evolve into malignant tumors. Malignant transformation has been proposed to occur as a consequence of the accumulation of genomic aberrations that successively overcome the cellular barriers to malignancy. Although a concerted effort has been made to discover the complement of molecular aberrations required for malignant transformation, the spectrum of events that must occur in a single cell, including which oncogene activation and tumor suppressor gene inactivation events can cooperate to induce malignant transformation, has remained elusive. A number of benign lesions without malignant potential accumulate genomic aberrations and have the potential to inform on the changes that are tolerated in benign tumors; however, they are insufficient to induce malignant transformation. In PNAS, Hafner et al. (1) comprehensively investigate the mutation status, mechanistic barriers to malignancy, and clonal relationship of seborrheic keratoses (SK), a tumor lineage that is without malignant potential.

Discussion

Fibroblast growth factor receptor 3 (*FGFR3*) and phosphatidylinositol 3-kinase catalytic subunit (*PIK3CA*) mutations have been previously identified in SK (2, 3). Hafner et al. (1) perform an extensive analysis of hot spot mutations in genes in the FGFR3–RAS–MAPK and PI3K–AKT pathways in 175 independent SK lesions from 25 SK patients. They showed a high frequency of *FGFR3* (71%), *PIK3CA* (50%), *KRAS* (20%), *EGFR* (7%), *HRAS* (3%), and *AKT* (2%) mutations in the benign tumors (1). A complete sequence analysis is likely to identify an even higher mutation rate, suggesting that most, if not all, SK lesions have at least one and likely several putative oncogenic mutations. Strikingly, despite their lack of malignant potential, 89% of SK had at least one mutation, and 45% had more than one mutation in a well-characterized oncogene. Mutations observed in SK are present in malignant tumors and when expressed in transgenic mice, are sufficient to induce tumors. Thus, a potent mechanism must constrain malignant conversion in SK. The frequency of comutations in *FGFR3* and *PIK3CA* was approximately that predicted by chance, suggesting that they are not coselected. In contrast, the frequency of comutations in *FGFR3* and *KRAS* or *PIK3CA* and *KRAS* was lower than predicted by chance, suggesting either a lack of selective pressure for both mutations to occur or a negative interaction between the consequences of each mutation.

Multiple lines of evidence, including their presence in SK, indicate that mutations in oncogenes are insufficient to induce malignancy. Patients with germline mutations in *FGFR3* that result in the same codon change as in somatic mutations found in SK do not develop malignant epithelial skin tumors at increased frequency (2). The authors show that the sites of mutations found at the highest frequency in SK are found at much lower frequency in malignant tumors and vice versa (1). Although a subset of the mutations found in SK exhibits lower transforming activity than those found more frequently in malignant tumors, many of these mutations do predispose to tumor development in animal models. The sites of *FGFR3* and *PIK3CA* mutations in SK are similar to those observed in precancerous and less aggressive forms of bladder and prostate cancers, compatible with a low malignant potential (3, 4). However, it is important to note that the frequency and spectrum of mutations in *PIK3CA* vary markedly across tumor lineages, and the mutations in *PIK3CA* observed in SK are located at hot spots that are seen recurrently in malignant tumors. Nevertheless, the mutations in cases where members of both the PI3K and RAS pathways are mutated were not sufficient to develop malignant tumors.

Hafner et al. (1) observe that, unlike many malignant tumors (5), SK is relatively genomically stable, and inactivating mutations in the tumor suppressors phosphatase and tensin homolog (*PTEN*), tuberous sclerosis 1 (*TSC1*), or *p53* are rare or absent. Thus, the malignant potential of oncogene mutations is likely lineage-dependent, interacting with intrinsic gene expression patterns, and it must occur in the context of other genomic aberrations, likely including loss of tumor suppressor function, to facilitate malignant transformation. Although the paucity of mutations in tumor suppressors and the relative genomic stability in SK likely provide a partial explanation for the lack of malignant transformation, further comprehensive analysis of SK both in terms of genomic events and functional consequences is warranted to identify the spectrum of mutations in this benign tumor and explore the mechanisms preventing malignant conversion.

To examine the functional consequences of the oncogene mutations in SK, Hafner et al. (1) examine phosphorylation of ERK and AKT. Compared with normal skin, SK showed significantly elevated levels of phosphorylated AKT. These findings are consistent with many tyrosine kinase receptors, which preferentially signal through the PI3K–AKT pathway, as opposed to RAS–RAF mutants, which signal primarily through the MEK–ERK pathway (6). It will be of interest to learn if SK harboring *KRAS* or *HRAS* mutations also exhibits a preferential signaling profile linked to ERK. The interaction of oncogene activation and tumor suppressor loss in malignant cells typically drives cellular proliferation and survival programs. The oncogene mutations in SK were not sufficient to alter the proliferation index, as measured by elevated Ki67 levels, or induction of apoptosis, as measured by activated caspase 3 levels. Thus, SK has the capacity to abrogate the expected functional outcomes of activation of RAS/ERK and PI3K/AKT pathways.

Senescence has been proposed to play a major role in preventing malignant transformation of melanocytic nevi (7). Indeed, nevi often harbor the same *BRAF* (V600E) mutation present in ~60% of malignant melanoma; however, melanocytic nevi rarely become malignant (8). BRAF (V600E) expression in mouse models results in induction of senescence in melanocytes, and senescent markers are highly expressed in human melanocytic nevi (9). In contrast, BRAF (V600E) expression in the setting of PTEN loss in murine models is sufficient to induce metastatic melanomas (10). Oncogene-induced senescence has been similarly observed with HRAS, KRAS, or AKT expression and can be overcome by loss of specific tumor suppressors (7). Using β-galactosidase activity and a set of senescence markers, Hafner et al. (1) fail to observe evidence of senescence in SK.

Author contributions: S.E.W. and G.B.M. wrote the paper.

The authors declare no conflict of interest.

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Further markers of accumulated DNA damage were also absent in SK. Thus, an as yet unexplained mechanism must constrain malignant transformation in SK.

Many possible mechanisms may account for the nonmalignant potential of cells apart from senescence, including inhibitory feedback signals, lack of a critical pattern of genetic aberrations, and epigenetic phenomena that cannot be determined by the techniques used in this study. Feedback mechanisms for the MEK–ERK and PI3K–AKT pathways have been described (11, 12). In addition, FGFR3 is sufficient to induce forkhead box protein N1 (FOXN1) expression and subsequent differentiation (13). FOXN1 expression caused squamous cell carcinoma cells to transition to a benign SK-like phenotype. An investigation into the epigenetic alterations within SK and other benign tumors, as well as the surrounding normal skin, may yield further clues as to the mechanisms that prevent malignant transformation.

Using X-chromosome inactivation, the study by Hafner et al. (1) analyzes independent SK lesions in eight heterozygous females and shows that 28 of 30 lesions with a single mutant oncogene and 18 of 19 with at least two mutations had selective X-chromosome inactivation supportive of clonality. However, although lesions with *FGFR3* and/or *PIK3CA* mutations tended to be in physical proximity, the mutations were frequently located at different sites in *FGFR3* and *PIK3CA*. Indeed, some patients had seven independent *FGFR3* nucleotide aberrations in different SK lesions. This, combined with the paucity of mutations in *PTEN*, *TSC1*, and *p53*, indicates a selective mechanism leading to accumulation of mutations, activation of FGFR3, and likely, activation of the other oncogenes targeted in SK. Thus, in cases where the original lesion was clonal, multiple subclones with independent *FGFR3* mutations must have developed into independent SK lesions. Intriguingly, in most cases, each SK lesion showed only a single *FGFR3* mutation, suggesting that each SK lesion likely represents the outgrowth of a dominant subclone from the original SK clone. An analysis of mutations at the single cell level will be required to ensure that the multiple mutations detected in an individual SK lesion occur in a single or different subclone.

Nonmalignant or low-grade urothelial tumors that derive from an epithelial structure related to skin may inform the findings of Hafner et al. (1). These tumors frequently harbor the same *FGFR3* mutations, tend to be genomically stable, and arise in a mulifocal manner (14). Two theories have been proposed to explain multifocality of urothelial cancers. The monoclonal theory holds that a single transformed cell proliferates and spreads throughout the urothelium by intraluminal implantation or intraepithelial migration. The oligoclonal theory holds that a carcinogen causes independent transformation of cells in different locations within the same tissue, resulting in genetically unrelated tumors. These two theories are not mutually exclusive, and it is possible that both phenomena can occur in the same patient (14). The study by Hafner et al. (1) seems to support the monoclonal model of synchronous multifocal tumor origin, although the few cases in which clonality was not identified could represent distinct clones. The observation that the same *FGFR3* and/or *PI3K* mutations tend to be observed in SK in physical proximity also supports a monoclonal explanation, and deviations may represent clones that have undergone subsequent genetic alterations. These findings raise the intriguing possibility that clonal SK cells may undergo intraepithelial migration within a field before emerging as visual tumors. Also of note, malignant urothelial tumors often show loss of the tumor suppressor *p53* (15), whereas SK failed to show loss of p53 function; this suggests that this alteration may be an essential hit lacking in SK, explaining the failure to acquire a malignant phenotype.

Angiomyolipomas, the most common benign tumors of the kidney, support the notion that tumor suppressor loss is an important precondition for metastatic competence. Angiomyolipomas can arise sporadically but are common features of lymphangiomyomatosis (LAM), which arises in patients with tuberous sclerosis. The proliferating smooth muscle cells within LAM associated with tuberous sclerosis are clones of the smooth muscle cells in renal angiomyolipomas (16). Angiomyolipomas harbor mutations in either *TSC1* or *TSC2*, whose protein products hamartin and tuberin inhibit the Ras homolog Rheb; this activates the mTOR within the target of rapamycin complex 1 (TORC1 complex). Loss of TSC1 or TSC2 function stimulates signaling through the TORC1 complex (17). Angiomyolipomas represent a curious example of a benign tumor that can distantly metastasize. The study by Hafner et al. (1) shows SK to lack TSC1 loss and loss of other tumor suppressors, which may contribute to the limited potential of SK to give rise to malignancy.

Conclusion

This report by Hafner et al. (1) represents a comprehensive approach to understanding the nature of benign tumors and may enhance our understanding of global genetic alterations that may be necessary, but are not sufficient, to result in malignancy. It also supports a model of SK tumor generation from single clones that have dispersed within an intraepithelial region. This study provides a basis for further investigation into the mechanisms by which tumors progress but may be blocked from becoming malignant.

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