The Conundrum of Genetic “Drivers” in Benign Conditions

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Abstract

Advances in deep genomic sequencing have identified a spectrum of cancer-specific passenger and driver aberrations. Clones with driver anomalies are believed to be positively selected during carcinogenesis. Accumulating evidence, however, shows that genomic alterations, such as those in \textit{BRAF}, \textit{RAS}, \textit{EGFR}, \textit{HER2}, \textit{FGFR3}, \textit{PIK3CA}, \textit{TP53}, \textit{CDKN2A}, and \textit{NF1/2}, all of which are considered hallmark drivers of specific cancers, can also be identified in benign and premalignant conditions, occasionally at frequencies higher than in their malignant counterparts. Targeting these genomic drivers can produce dramatic responses in advanced cancer, but the effects on their benign counterparts are less clear. This benign-malignant phenomenon is well illustrated in studies of \textit{BRAF} \textit{V600E} mutations, which are paradoxically more frequent in benign nevi (~80%) than in dysplastic nevi (~60%) or melanoma (~40%-45%). Similarly, human epidermal growth factor receptor 2 is more commonly overexpressed in ductal carcinoma in situ (~27%-56%) when compared with invasive breast cancer (~11%-20%). \textit{FGFR3} mutations in bladder cancer also decrease with tumor grade (low-grade tumors, ~61%; high-grade, ~11%). “Driver” mutations also occur in nonmalignant settings: \textit{TP53} mutations in synovial tissue from rheumatoid arthritis and \textit{FGFR3} mutations in seborrheic keratoses. The latter observations suggest that the oncogenicity of these alterations may be tissue context-dependent. The conversion of benign conditions to premalignant disease may involve other genetic events and/or epigenetic reprogramming. Putative driver mutations can also be germline and associated with increased cancer risk (eg, germline \textit{RAS} or \textit{TP53} alterations), but germline \textit{FGFR3} or \textit{NF2} abnormalities do not predispose to malignancy. We discuss the enigma of genetic “drivers” in benign and premalignant conditions and the implications for prevention strategies and theories of tumorigenesis.

Cancers are known to arise, at least in part, as a result of acquired changes in DNA, and such changes accumulate over time (1). With the advent of next-generation sequencing (NGS), the full complement of genetic alterations in a given cancer can be identified (2). Some of these abnormalities are “passengers” that do not drive progression to metastatic disease (3). Other alterations, termed genetic “drivers” (4,5), are implicated in pathways crucial to the ability of cancer cells to grow and survive. Clones harboring driver anomalies are presumed to be positively selected in the evolution of neoplasia to invasive and advanced cancer (3). Overall, a basic premise in oncology is that, via the process of clonal selection, driver mutations are rare in benign conditions, variably present in premalignancy (depending on severity and cancer risk), and most frequent in advanced cancer (3,6). Recent studies, albeit limited, have suggested that genetic drivers can occur in early cancer (7) and premalignancy (8–18). The identification of genetic drivers has resulted in the development of targeted therapies with promising outcomes in patients with advanced cancers that harbor actionable
alterations (19). In this review, we describe an emerging body of literature indicating that genomic drivers, considered a hallmark of specific cancers, can also be found in benign conditions and in premalignant lesions, sometimes at frequencies higher than in the corresponding tumors (Table 1), and discuss the implications of these findings for current theories of carcinogenesis and designing prevention strategies.

**BRAF**

BRAF is a member of the RAF kinase family, with CRAF being the more ubiquitously expressed component in physiologic mitogen-activated protein (MAP) kinase pathway signaling (20,21). MAP kinase signaling plays a key role in cell division, differentiation, and survival.

**BRAF in Cancer**

Mutations in the BRAF gene, especially the V600E mutation, lead to constitutive activation of the MAP kinase pathway and increase in growth signals (22). While BRAF V600E mutations are present in almost half of patients with melanoma (22–25), they are also found in up to 17% of patients with diverse, advanced metastatic cancers seeking therapy following failure of standard treatments (19). BRAF mutations are clinically important targets because they are sensitive to BRAF (26–31) or MAP kinase-ERK kinase (MEK) inhibitors (32).

The BRAF V600E mutation has been identified in 40% to 45% of metastatic melanomas in prospective studies with comprehensive interrogation (23,24). This mutation is considered an oncogenic driver, and melanomas bearing these BRAF V600E alterations have excellent responses to BRAF inhibitors such as vemurafenib or dabrafenib (26,27); these responses are entirely comparable with the most effective single-agent, oncogene-targeted therapies in non–small lung cancer (EGFR and ALK inhibitors) (33–36).

**BRAF in Melanocytic Nevi and Dysplastic Nevi**

The BRAF V600E mutation is very common in benign melanocytic nevi, being discernible in 70% to 88% of such lesions (37,38). It is also found in approximately 60% of dysplastic nevi, with no clear correlation with degree of dysplasia (13); the latter can be a precursor of melanoma. Thus, BRAF mutations are considered to be one of the earliest events in melanoma development (39). Curiously, however, less than 0.03% of melanocytic nevi (40) and only about 4.8% of dysplastic nevi transform to melanoma (although risk increases with the number and size of dysplastic nevi and degree of dysplasia) (41). As mentioned, the frequency of BRAF V600E mutations is paradoxically lower (40%-45%) (22–25) in melanoma compared with melanocytic nevi (37,39) or dysplastic nevi (13) (Table 1). If BRAF mutations were a strong driver for melanoma development, one would assume they would be more commonly seen in patients with melanoma than in its benign counterpart. Because approximately 50% of patients with melanoma with BRAF mutations respond to BRAF (26–31) or MEK inhibitors (32), there is no doubt that these melanomas depend on the MAP kinase pathway, but whether oncogenic BRAF itself is sufficient to sustain oncogenic MAP kinase signaling is an open question.

A variety of changes have been reported in preexisting, previously stable melanocytic nevi in patients receiving BRAF inhibitors, including increased pigmentation and involution. For instance, Haenssle et al. (42) observed that the BRAF inhibitor vemurafenib could lead to dynamic changes in melanocytic nevi, with both regression or increased size or pigmentation of melanocytic nevi at different sites. The biochemical correlates of these effects have not been elucidated. However, it may be that even in dormant melanocytic nevi BRAF inhibitors increase expression of microphthalmia-associated transcription factor (MITF), with several consequences including upregulation of pigmentation pathways. In contrast, a melanoma with a BRAF V600E mutation in the same individual regressed with therapy (43), suggesting the enigmatic conclusion that the BRAF V600E mutation in melanoma is a driver while it is not a driver in melanocytic nevi. Furthermore, Cohen et al. observed development of new dysplastic nevi or new primary melanomas when patients were treated with vemurafenib for melanomas with BRAF V600E mutations (43). Some melanocytic nevi that were newly developed or had morphological changes observed in patients with melanoma receiving BRAF inhibitor therapy for metastatic BRAF V600E-disease have activating NRAS mutations (not BRAF) and are thought to arise as a consequence of the well-described triggering effect that these agents can have on RAS-activated premalignant lesions (44,45).

Despite the dynamic changes described in some benign melanocytic nevi on BRAF inhibitor therapy, for the most part these nevi remain static when a patient is treated with vemurafenib or dabrafenib, even though the vast majority of these nevi harbor BRAF V600E “driver” mutations. This dichotomy is difficult to reconcile, but it is possible to postulate that an endogenous inhibitor against BRAF or other constituents of the MAP kinase pathway may exist in melanocytic nevi, hence protecting melanocytic nevi from behaving like a malignancy and from regressing in the face of BRAF inhibitor treatment (tumor-suppressor hypothesis). Alternatively, or in addition, one of more cofactors may need to be mutated in order for transformation of melanocytic nevi to melanoma to occur (second oncogene hypothesis) (Figure 1). In support of both theories, investigation of activated ERK in melanocytic nevi by immunohistochemistry consistently demonstrates low levels of MAP kinase pathway activation (whereas it is uniformly elevated in BRAF V600E metastatic melanomas) (46).

In an effort to clarify possible BRAF codrivers, experimental models have been generated with genetic manipulation of tumor suppressors known to be altered in invasive and metastatic melanomas. Patton et al. (47) showed that BRAF V600E-mutated zebrafish developed patches of ectopic melanocytes; however, when TP53 was also deleted, zebrafish rapidly developed invasive melanomas. Using the same BRAF V600E-mutant/TP53-deficient zebrafish model, Kaufman et al. recently demonstrated that the crestin gene is not only expressed in neural crest progenitors (during the embryonic state) but also re-expressed during melanoma development, suggesting that the crestin gene is necessary for melanomagenesis. Overexpression of SOX10 (the neural crest master transcription factor) in melanocytes accelerated the onset of melanoma, indicating that a dedifferentiated, epigenetic state may also play a part in cancer transformation following the appearance of driver genetic alterations (48). In mouse models in which BRAF V600E was conditionally expressed in melanocytes, melanocytic proliferations were observed with histologic features of melanoma, but invasive melanomas did not develop (49). In two different models, codelletion of PTEN or CDKN2A with expression of BRAF V600E produced highly penetrant invasive and even metastatic melanomas (49,50).
| Gene       | Aberration            | Benign or premalignant condition: % with aberration (references) | Malignant condition: % with aberration (references) | Examples of specific mutations found in both benign/premalignant conditions and cancer |
|------------|-----------------------|---------------------------------------------------------------|-------------------------------------------------|----------------------------------------------------------------------------------|
| BRAF       | Mutations             | Melanocytic nevi: 70%-88% (37,38) Dysplastic nevi: ~60% (13) | Melanoma: ~40%-45% (22–25) Across cancers: Up to 17% (19) | V600E                                                                            |
| NRAS*      | Mutations             | Melanocytic nevi: 6%-14% (71,72) Includes Q61K Congenital melanocytic nevi: 70%-95% (73,74) Includes Q61K and Q61R | Cutaneous melanoma: 18%-28% (62,64) Includes Q61 | Across cancers: Up to 8% (19)                                                    |
| GNAQ†      | Mutations             | Sturge-Weber syndrome: R183Q; 88% (83)                         | Uveal melanoma: Q209L: 22%-45% R183Q: 3%-6% (84) | R183Q                                                                            |
| FGFR3†     | Mutations             | Seborrheic keratosis: ~18%-85% (102–105) Includes R248C, S249C, G372C, S373C, A393E, K652E, K652M | Cervical cancer: 1.7-25% (95,99) Includes S249C | R248C, S249C, G372C                                                            |
| PIK3CA     | Mutations             | Seborrheic keratosis: ~16% (103) Includes E542K, E545K, H1047R Epidermal nevi: ~27% (103) Includes E545G Fibroadipose hyperplasia: 90% (118) Includes H1047R and H1047L | Melanoma: ~1%-3% (120,121) Includes E542K, E545K, H1047R Across cancers: 9%-18% (19,114,115,123) Includes E542K, E545K, E545G, H1047R | E542K, E545K, E545G, H1047R                                                     |
| ALK        | Rearrangement         | Inflammatory myofibroblastic tumor: ~50% (129) Includes TPM3-ALK, TPM4-ALK | Anaplastic large cell lymphoma: 60%-80% (130) Includes NPM-ALK, TPM3-ALK Adenocarcinoma of lung: 3%-7% (130) Includes EML4-ALK | TPM3-ALK                                                                         |
| NOTCH 1    | Mutations             | Sun exposed skin: ~20% (176) Includes P168L, R353C, H468L, D464N, G394S, R176W, W1768*, P1770S, Q192*  | Cutaneous squamous cell carcinoma: ~75% (192) Includes R353C, C423F, Q610*, W1768*, P1770S, Q192* | R353C, W1768*, P1770S, Q192*                                                    |
| TP53*      | Mutations             | Rheumatoid arthritis: 17-46% (137,138) Includes R177S, Q192L, R196*, K138, H193Y, E224fs, N239S | One of the genes with highest mutation rate across tumors: ~40% (134,136) Includes H193Y, E224fs, N239S | H193Y, E224fs, N239S                                                             |
| NF1*       | Mutations             | Neurofibromas and pilocytic astrocytomas (in setting of NF1 germline mutations) (78) | Glioblastoma:15%-18% (156,157) Melanoma:13% (158) Associated with inactivating mutation or loss | K4N, G197C, R200fs                                                              |
| NF2†       | Mutations             | Schwannomas, meningoïdia, glioma and ependymoma astrocytomas (in setting of NF2 germline mutations) (168) | Mesothelioma: 30%-50% (166) Hepatocellular carcinoma: 23% (166) Anaplastic thyroid carcinoma: 18% (166) |                                                                                   |
| HER2 protein Overexpression | Ductal carcinoma in situ: ~27-56% (8,12,18) | Invasive breast cancer: ~11%-20% (85,86) | Not applicable                                                                 |                                                                                   |

*Germline syndromes with increased cancer risk. ALK = anaplastic lymphoma receptor tyrosine kinase; BRAF = B-Raf proto-oncogene, serine/threonine; FGFR3 = fibroblast growth factor receptor 3; GNAQ = guanine nucleotide binding protein, q polypeptide; HER2 = human epidermal growth factor receptor 2; NF1 = neurofibromin 1; NF2 = neurofibromin 2; NRAS = neuroblastoma RAS viral oncogene homolog; FIK3CA = phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; TP53 = tumor protein p53. †Sturge Weber is because of GNAQ mutations that appear as a result of a postzygotic mutation, and hence patients have somatic mosaicism—the mutation is found in some but not all body cells. ‡Germline syndromes without increased cancer risk.
Another phenomenon of interest in unraveling some of these questions is the intriguing observation that stably transducing the “oncogene” BRAF V600E into melanocytes leads to blockage of cellular proliferation and to an increase in cellular senescence, despite phosphorylation of the downstream effector ERK (51). This finding contrasts with the transforming effect of BRAF V600E when introduced into p16-null melanocytes (p16 being one of two tumor suppressor gene products of CDKN2A) (50). This phenomenon has been designated “oncogene-induced senescence” (reviewed elsewhere [52–56]). The presence of insulin-like growth factor binding protein 7 (IGFBP7) was initially considered to be involved in mediating oncogene-induced senescence in melanocytic nevi and melanoma (57), but this observation has not been validated (51,58). A genome-wide investigation of differentially expressed genes in BRAF V600E melanocytic nevi compared with melanocytic nevi with BRAF wild-type revealed consistent upregulation of CDKN2A, CDKN1C, and MITF (59). Each of these is an intriguing candidate for maintaining the senescent state, but 20 distinct pathways were noted to be differentially expressed in this analysis and a functional dissection of these pathways has not been performed (59). Unbiased, genome-wide shRNA library screening for genes capable of reversing BRAF V600E-induced senescence identified PTEN as the most potent, and pharmacologic inhibition of PI3K signaling restored p16INK4B-dependent senescence (49). The same group identified seven genes that, when silenced, could revert BRAF-associated senescence (60). However, only RAS and RASEF were differentially expressed in melanocytic nevi vs melanoma. Functional studies suggested that RASEF acts in a Rb-dependent fashion and affects expression of the senescence-associated secretome (60).

It remains puzzling that BRAF mutations are less commonly seen in melanoma than in melanocytic or dysplastic nevi. Recent descriptive and functional analysis suggest that alterations of CDKN2A/B, PTEN, CDK4, CCND1, ERBB4, and AKT, known to occur in human melanomas with high frequency, facilitate relief of oncogene-induced senescence, leading to selection of these melanocytic nevi during malignant evolution (15,16,61). However, the cooperation of these aforementioned alterations with BRAF V600E to hyperactive the MAP kinase pathway has not been established. Working out this mechanistic connection is essential to defining the most rational preventive strategy to explore in preclinical models and, eventually, in clinical trials. It remains possible that direct BRAF inhibition would be effective as a strategy to impede the ability of a cofactor to generate constitutive MAP kinase pathway signaling. Understanding the entire pathway that ties these phenomena together will provide other candidate points of intervention that could be more amenable to safe and effective use in the prevention setting.

RAS

RAS in Cancer

Three RAS genes (KRAS, NRAS, and HRAS) are amongst the most frequently altered oncogenes identified in human cancers. Mutations in these genes activate several downstream pathways, depending on the mutation type and subtype (62–64).

The frequency of aberrations in each RAS gene differs depending on the underlying malignancy. KRAS (notably G12 and G13 mutations) is commonly mutated in patients with diverse malignancies such as pancreatic ductal adenocarcinoma (71%-98% of
cases), colorectal adenocarcinoma (35%-45%), and lung adenocarcinoma (19%-31%) (19,62,64). NRAS (notably Q61) aberrations have been associated with several tumors including melanoma (18%-28%), multiple myeloma (20%), and thyroid carcinoma (9%) (19,62,64). HRAS (G12, G13 and Q61) mutations are not as frequent when compared with KRAS or NRAS mutations; however, they have been reported in various cancer types including bladder urothelial carcinoma (1%-6%), head and neck squamous cell carcinoma (5%), and thyroid carcinoma (4%) (62,64). RAS mutations confer resistance to EGFR inhibitors in advanced colorectal and non-small cell lung cancers (65,66). To date, there are no effective therapies to directly overcome RAS mutations although multiple studies are ongoing, including application of agents targeting signals downstream of RAS (eg, MEK), screening compounds for synthetic lethality, identifying drugs that effectively interfere with RAS membrane localization, and the development of direct RAS inhibitors (62).

**RAS in Premalignancy**

For certain cancers, oncogenic RAS drives early neoplastic development and progression. Examples include progression of pancreatic premalignancy (intraepithelial neoplasia) driven by KRAS mutation, followed by inactivation of tumor suppressor genes CDKN2A and finally the inactivation of TP53 and deletion of SMAD4 (67,68). Similarly, the development of the colorectal adenocarcinoma neoplastic sequence is also attributed to a stepwise accumulation of mutations identified in premalignant adenomas, including those in the APC, KRAS, and TP53 genes (69,70).

**RAS in Benign Conditions**

Although NRAS mutations are not as frequent when compared with the example of BRF mutations (found in the majority of melanocytic nevi), the NRAS Q61K mutation is discerned in 6% to 14% of benign melanocytic nevi (71,72). Considering that the presence of NRAS mutation in melanoma is somewhat higher (18%-28%) (62,64) than in melanocytic nevi, it is speculated that NRAS mutations are one of the driver mutations for melanoma development. Interestingly, NRAS mutations (Q61K or Q61R) are detected in 70% to 95% of cases of congenital melanocytic nevi (benign melanocytic proliferation that develops in utero), which have a 465-fold increase risk of developing melanoma (73–75). NRAS mutation alone was not a strong driver for melanoma development in a melanoma mouse model, suggesting that additional mutations, eg, loss of CDKN2A, are required (76). The higher melanoma risk observed in patients with congenital melanocytic nevi suggests that length of time that NRAS mutations are harbored may be a factor in susceptibility to cancer development.

**RAS Germline Mutations**

Congenital RAS mutations are associated with RASopathies, which are a group of genetic developmental disorders affected by increased RAS-MAPK signaling. Although full detail of RASopathies is beyond the scope of this review and has been extensively described elsewhere (77–80), some of the syndromes associated with germline mutations in this pathway include Noonan syndrome (PTPN11 and KRAS mutations), LEOPARD syndrome (PTPN11 and RAF1 mutations), neurofibromatosis type 1 (NF1 mutation), Costello syndrome (HRAS mutation), and cardio-facio-cutaneous syndrome (BRAF, MAP2K1 and 2, KRAS mutations). Each RASopathy has unique clinical presentations; however, because of the common underlying mechanisms with increased RAS-MAPK signaling, they share overlapping characteristics with nonmalignant conditions such as craniofacial dysmorphology, cardiac malformation, cutaneous and musculoskeletal abnormalities, and neurocognitive impairment.

Importantly, patients with RASopathies do have an increased risk of malignancy, ranging from 1.6% to 15%, which manifests during early childhood and adolescence (77,81,82). Malignancies associated with RASopathies differ from syndrome to syndrome with some overlap. Neurofibromatosis 1 has been associated with malignant peripheral nerve sheath tumors (MPNSTs), pheochromocytomas, rhabdomyosarcomas, and gastrointestinal stromal tumors (GISTs) (78); Costello syndrome, with an increased risk of rhabdomyosarcoma, neuroblastoma, and transitional cell carcinoma (77); and Noonan syndrome, with acute lymphoblastic leukemia, neuroblastoma, and rhabdomyosarcoma (82).

**GNAQ**

GNAQ-activating mutations lead to an increase in MAP kinase pathway signals. Interestingly, they are found in about 88% of Sturge-Weber syndrome (GNAQ R183Q). This syndrome is characterized by specific clinical features such as port-wine stains affecting the skin, as well as leptomeningeal vascular malformations. An increased risk of malignancy has not been documented (83).

Mutations in GNAQ are also found in patients with cancer. For instance, GNAQ alterations are frequent in uveal melanoma, with a minority (3%-6%) harboring R183Q, while GNAQ Q209L is detected in 22% to 45% of patients (84) (Table 1).

The reason that Sturge-Weber patients do not develop cancer may be because the GNAQ mutations they harbor develop as a consequence of somatic mosaicism and, hence, are not found in all body cells. (Somatic mosaicism occurs as a result of a post-zygotic mutation and refers to the occurrence of two genetically distinct populations of cells within an individual. In contrast to classic inherited mutations, somatic mosaic alterations may affect only a portion of the body and are not transmitted to children.) Hence, mutations in GNAQ may require specific tissue contexts to produce cancer.

**HER2**

**HER2 in Invasive Breast Cancer vs Ductal Carcinoma In Situ**

HER2 can be overexpressed, amplified, or, less commonly, mutated in a variety of cancers (85,86). Treatment with anti-HER2 agents is US Food and Drug Administration (FDA)–approved for breast and gastric cancers and has activity in other malignancies as well (87).

Ductal carcinoma in situ (DCIS) is the most common type of premalignant noninvasive breast cancer (88). Although HER2 overexpression is implicated in the pathogenesis of breast cancer (89–91), HER2 is more commonly overexpressed in patients with DCIS (~27%-56%) (8,12,18) when compared with invasive breast cancer (~11%-20%) (85,86) (Table 1). The underlying reason why HER2, which is considered to be an important driver of breast cancer (91), is more highly expressed in noninvasive disease, is unclear. Recent clinical trial data illustrate the
complexity of HER2 status in DCIS and invasive breast cancer and suggest that ERBB2 status in DCIS can be both prognostic and predictive. Increased ERBB2 mRNA expression is correlated with risk of ipsilateral DCIS but not invasive breast cancer. Radiation therapy was more effective in HER2 overexpressing DCIS, both in reducing ipsilateral DCIS and invasive breast cancer (92). However, there is no clinical data on the efficacy of HER2-targeted therapy by HER2 expression status in DCIS.

**FGFR3**

**FGFR3 in Cancer**

Fibroblast growth factor receptor 3 (FGFR3) belongs to a family of tyrosine kinase receptors, and its oncogenic role has been proposed as a mechanism underlying the development and progression of cancer (93). FGFR3 aberrations are found in multiple tumor types, most commonly being described in urothelial bladder cancer and some reports in cervical cancer (94–99). Aberrations include FGFR3 R248C, S249C, and G372C in cancer (94–99) (Table 1). These mutations result in the FGFR3 kinase enzyme becoming locked in the activated position, leading to ligand-independent signaling activation (97,98,100). However, the frequency of FGFR3 mutations in bladder cancer paradoxically decreases with advanced and higher-grade tumors (frequency of FGFR3 mutations in TaG1 tumor: ~61%, TaG2: ~58%, TaG3: ~34%, T1G3: ~17%, T2-T4, high-grade tumors: ~11%) (94,101). Although similar literature for cervical cancer in this regard is lacking, it requires further investigation.

**FGFR3 in Benign Conditions**

FGFR3-activating mutations have been reported in 18% to 85% of seborrheic keratoses; these are benign lesions with no risk of cancer (102–105) (Table 1). The wide range of frequency of FGFR3 mutations may be because of different patient characteristics, locations of the seborrheic keratoses that were biopsied (seborrheic keratoses from head and neck had a lower frequency of FGFR3 mutations when compared with the ones from the trunk and extremities (102)), and experimental designs. Similarly, 33% of epidermal nevii harbor FGFR3 R248C mutation (106). Some of the specific FGFR3 mutations, including R248C, S249C, and G372C alterations, are also found in urothelial bladder and cervical cancers while others are not (Table 1) (94–97,99). Functional studies of FGFR3 mutations in benign skin conditions are limited. Hafner et al. (107) evaluated keratinocyte cell lines transduced with either FGFR3 wild-type or the R248C mutation and showed that FGFR3-mutant keratinocytes had lower levels of apoptosis and enhanced phosphorylation of ERK1/2 when compared with wild-type controls. However, in the same in vitro model, FGFR3 mutations did not alter migration and senescence when compared with wild-type keratinocytes. Furthermore, in clinical samples of seborrheic keratosis, there were no differences in Ki-67 expression between wild-type and FGFR3-mutant samples, suggesting that FGFR3 may not contribute to proliferation in seborrheic keratosis (102,107).

**FGFR3 Germline Mutations**

FGFR3 germline mutations have an inhibitory impact on bone growth that leads to dwarfism syndromes, with mutations identical to those discerned in some cancers and seborrheic keratoses (eg, R248C and S249C) (108–110). FGFR3 inhibitor exposure restored dwarf mice harboring FGFR3 mutations to a normal phenotype (111). Although patients with dwarfism syndromes have decreased life expectancy (average 10 years shorter), mainly because of heart disease–related mortality (112), these patients have no documented increased incidence of cancer or of seborrheic keratoses.

**PIK3CA**

**Mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA)** are found in diverse malignancies (113). Tumors harboring PIK3CA mutations may respond to PI3K/Akt/mTOR inhibitors (19,114,115), confirming the PIK3CA oncogenic role. PIK3CA mutations are also detected in a subset of benign epidermal nevi and seborrheic keratoses (103) (Table 1).

**PIK3CA in Cancer**

PIK3CA is part of the PI3K signaling pathway that is crucial for cell growth and survival (116). Certain PIK3CA anomalies, including E542K, E545K, and H1047R mutations, have been found in multiple cancer types including colon, breast, and bladder cancer (113). It has been reported that PIK3CA mutations predict response to treatment with combination regimens that include a PI3K/AKT/mammalian target of rapamycin (mTOR) inhibitor (19,114,115). Further relevance of PIK3CA as a therapeutic target has recently been gleaned from phase I/II trials with PI3K alpha selective inhibitors. Three such agents produce highly comparable results. Notably, these trials also demonstrated that the response rate with these agents as monotherapy is lower than that observed with BRAF, EGFR, and ALK inhibitors in their respective genotype-defined populations in melanoma and non–small cell lung cancer. Furthermore, emergence of clones that are wild-type for PIK3CA in progressing tumors (117) highlights the variable role of PIK3CA across cancers. Taken together, the data suggests that targeting PIK3CA alone may be less useful than in combination regimens, consistent with the presence of codrivers in tumors with abnormalities in this gene.

**PIK3CA in Benign and Premalignant Conditions**

Similar to the FGFR3 and BRAF mutation story, PIK3CA-activating alterations are implicated in the pathogenesis of seborrheic keratoses (~16%) and epidermal nevi (~27%), with mutations identical to those found in certain malignancies (includes E542K, E545K, and H1047R) (103). PIK3CA-activating alterations are also reported in patients with fibroepithelial hyperplasia, which is a syndrome of nonmalignant progressive segmental overgrowth of fibrous and adipose tissue, where 90% of patients were found to have either PIK3CA H1047R or H1047L mutations (118), and in hemimegalencephaly (HME), characterized by nonmalignant overgrowth of either one of the two cerebral hemispheres, as well as other brain malformations (119). Although PIK3CA mutations are uncommon in melanoma (~1%-3%) (120,121) or basal cell and squamous cell carcinoma of skin (122), they are found in 9% to 18% of other solid tumors (19,114,115,123) (Table 1). In the context of the PI3K/Akt/mTOR pathway, AKT1-activating mutations, which are found in diverse malignancies (124), are also reported in seborrheic keratoses (E17K: 2%) (125). Lastly, the PI3K/Akt/mTOR pathway, an
important driver in lung premalignancy, could be reversed by an inhibitor of this pathway (126).

**ALK**

Fusion of ALK with different genomic partners leads constitutively to activation and drives tumorigenesis (127). The ALK inhibitors crizotinib and ceritinib are very effective and have been FDA-approved in lung adenocarcinoma with ALK rearrangement (35). Interestingly, ALK rearrangement is also reported in patients with benign inflammatory myofibroblastic tumors (128,129) (Table 1).

**ALK in Cancer**

ALK rearrangement has been described in a variety of tumors, including anaplastic large cell lymphoma (NPM-ALK: 60%-80%, TPM3-ALK: 12%-18%), lung adenocarcinoma (EML4-ALK: 3%-7%), and breast cancer (EML4-ALK: 0%-2.4%) (130). ALK inhibitors have successfully prosecuted ALK-altered tumors (35,131,132).

**ALK in Benign Tumors**

Interestingly, about half of inflammatory myofibroblastic tumors harbor rearrangements in the ALK locus (TPM3 or TPM4-ALK), leading to aberrant ALK signaling (129). Inflammatory myofibroblastic tumors (also called inflammatory pseudotumors) occur in different anatomic locations including lung, liver, breast, soft tissue, and colon (133). Although locally destructive forms of this disease have been reported, metastases are uncommon, with a less than 5% risk (133). Therapies include resection or observation, and, if necessary, chemotherapy and radiation have been used (133). ALK inhibition with crizotinib has been evaluated and shown to have clinical benefit (128).

**TP53**

The TP53 tumor suppressor gene is amongst the most frequently mutated in cancer, being reported in 40% to 50% of human malignancies (134–136). Despite the strong association between TP53 abnormalities and malignancy, mutations in this gene have also been described in benign diseases as below (137,138) (Table 1).

**TP53 Mutations in Cancer**

TP53 mutations can result in both gain of function and loss of function; the latter is mostly associated with a dominant-negative effect over wild-type p53 (139). Mutations are especially frequent in serous ovarian (95% of patients) and serous endometrial carcinomas (89%) (134). Furthermore, patients with germline TP53 mutations have Li-Fraumeni syndrome that is associated with a high incidence of cancer (140).

**TP53 Mutations in Synovium of Rheumatoid Arthritis**

Rheumatoid arthritis is linked to synovial tissue hyperplasia but is not reported to be a direct risk for cancer development. It has been documented that 17% to 46% (137,138) of synovial tissues from patients with rheumatoid arthritis had alterations in TP53 that are identical to the mutations seen in malignancies (eg, H193Y, E224fs, N239S); these mutations were not observed in patients with osteoarthritis (138). Even so, Reme et al. (137) followed four patients with TP53-mutated rheumatoid arthritis for two years and none developed signs of malignancy. Further, synovial sarcoma is not a complication of rheumatoid arthritis. These data indicate that the oncogenic role of mutated TP53 is tissue-context dependent.

Although the role of TP53 mutation in the pathogenesis of rheumatoid arthritis remains to be fully clarified, in vitro and in vivo work shows that inactivation of TP53 in synovial cells leads to stimulation of metalloproteinase and cytokines, which are in turn associated with enhanced proliferation and invasiveness of synoviocytes (141). Further, Angelo and colleagues (142) reported that mutated TP53 leads to interleukin-6 (IL-6) overexpression, at least in part by modulating specific transcription factor binding to the IL-6 promoter. These observations are of special interest because IL-6 is a potent proinflammatory agent that plays a critical role in the pathogenesis of inflammatory disease and is important for both joint destruction and systemic manifestations of rheumatoid arthritis (143). Furthermore, tocilizumab, which binds the IL-6 receptor, is FDA-approved for treatment of active, moderate to severe rheumatoid arthritis (and systemic juvenile idiopathic arthritis) (144).

**TP53 Germline Mutations**

Mutations in germline TP53 are associated with the Li-Fraumeni syndrome, a rare disorder that greatly increases the risk of developing several types of cancer (breast cancer, sarcomas, brain tumors, leukemias, and adrenocortical carcinomas), particularly in children and young adults (140).

**CDKN2A**

**CDKN2A in Cancer**

Aberrations in cyclin-dependent kinase (CDK) pathways disrupt cell cycle restriction and contribute to genomic instability and tumor proliferation (145,146). Cyclin D1 forms complex with CDK4/6, leading to Rb protein phosphorylation and subsequent G1 cell-cycle progression mediated by E2F transcription factor. This pathway is regulated by inhibitors including CDKN2A (p16) and/or CDKN2B (p15) (147). Loss or inactivating mutations of CDKN2A can lead to alteration in CDK pathway and tumorigenesis. Aberrations in CDKN2A (mostly loss or hypermethylation) have been observed in about 19% of patients with diverse malignancies and have been associated with poor clinical outcome (148,149).

**CDKN2A in Premalignant Conditions**

Aberration in CDKN2A is observed in multiple premalignant conditions, including pancreatic intraepithelial neoplasia (42%) (17), colorectal adenomas (34%) (10), and Barrett’s esophagus (33%) (9). However, Shain et al. recently reported that aberration of CDKN2A was a late event in melanoma development, exclusively observed in patients with invasive melanoma and not associated with precursor skin lesions that were commonly associated with BRAF or HRAS aberrations (16). The latter observations suggest that CDKN2A aberrations are critical for melanoma development (Figure 1).
CDKN2A Germline Mutations

CDKN2A germline mutations are associated with familial atypical multiple mole melanoma (FAMMM) syndrome, which is an autosomal dominant condition characterized by high numbers of dysplastic nevi (>50) and a history of melanoma in one or more first- or second-degree relatives (150,151). CDKN2A germline mutations increase the risk of melanoma (152,153) and pancreatic cancer (154), and possibly sarcoma, breast, and esophageal cancers (155).

NF1

NF1 in Cancer

Neurofibromin 1 (NF1) is a tumor suppressor gene that encodes RAS GTPase-activating protein (GAP) (78). NF1-inactivating mutations or loss lead to hyperactivation of RAS and increase downstream effector signaling such as that by PI3K/Akt/mTOR and MAP kinase (62).

Somatic mutations of NF1 (inactivating mutations or loss) are identified in a variety of sporadic cancers, including glioblastoma (15%-18%) (156,157), melanoma (~13%) (158), and lung adenocarcinoma (~7%) (159). NF1 mutations are considered to be mutually exclusive with NRAS or BRAF mutations, suggesting their dedicated role in the RAS-MAPK pathway (78,158). Preclinical studies suggest that NF1 mutations are potentially targetable with mTOR inhibitors such as everolimus (160) or MEK inhibitors such as trametinib (161), or by combining both mTOR and MEK inhibitors, and that suppression of signals downstream of RAS with MEK and/or mTOR is capable of controlling tumorigenesis (162,163).

NF1 Germline Mutations

Neurofibromatosis type 1, an autosomal dominant disorder caused by NF1-inactivating mutations (164), can include cutaneous, neurologic, optic, and orthopedic abnormalities and benign and malignant tumors (78,164). Many tumors associated with neurofibromatosis type 1 originate from the neural crest cell (pheochromocytomas, neurofibromas, plexiform neurofibromas, and malignant peripheral nerve sheath tumors) but can also be derived from neuroepithelial cells (pilocytic astrocytomas); there are also non-neural crest-derived tumors such as rhabdomyosarcoma and gastrointestinal stromal tumors (78).

Neurofibromatosis type 1-associated cancers can demonstrate various degrees of aggressiveness, even though they may arise from the same cell types. For example, in considering lesions that derive from the neural crest cell, cutaneous neurofibroma are typically benign and do not transform; plexiform neurofibroma can invade adjacent tissue but remain nonmetastatic while malignant peripheral nerve sheath tumors are highly aggressive with strong metastatic potential (78). To date, it is not entirely understood why different degrees of aggressiveness can develop in tumors of similar origin in patients with neurofibromatosis type 1. Mouse models with neurofibroma and malignant peripheral nerve sheath tumors showed both conditions had sustained ERK activation (165), suggesting that other molecular factors may be responsible for cancer aggressiveness. Identifying such factors may lead to better therapeutic targeting for patients with neurofibromatosis type 1 and sporadic cancers.

NF2

NF2 in Cancer

The Neurofibromin 2 (NF2) gene is a tumor suppressor that encodes a protein known as merlin, which affects diverse cell signaling pathways (166,167). Notably, merlin inhibits mTORC1 and the complex formation of Src/FAK, thus controlling PI3K and RAS-MAPK signaling pathways, respectively (166,167). Thus, inactivating mutations of NF2 lead to increases in PI3K and RAS-MAPK signaling.

Somatic mutations in NF2 genes have been found in diverse cancer types, including mesothelioma (30%-50%), hepatocellular carcinoma (~23%), and anaplastic thyroid carcinoma (~18%) (166). These malignancies are, however, not increased in patients with germline NF2 mutations. Not surprisingly, somatic mutations in the NF2 gene are also identified in some sporadic tumors that are associated with neurofibromatosis type 2, including schwannoma (42%), meningioma (31%), glioma (27%), and ependymoma (4%) (166).

NF2 Germline Mutations

Neurofibromatosis type 2, an autosomal dominant disorder (168), is characterized by the development of benign tumors including schwannomas (notably vestibular schwannomas), meningioma, and low-grade central nervous system neoplasms (glioma and ependymoma) (168). As mentioned, some sporadic cases of these neoplasms harbor somatic NF2 mutations (166). However, to date, there is no data that suggests that patients with neurofibromatosis type 2 are predisposed to an increased risk of malignancies (166,168). This observation is perplexing. It has been postulated that the lack of a vulnerability to an increased incidence of malignant tumors in individuals with neurofibromatosis type 2 could be because of the type of mutations found in the germline vs the somatic settings. Indeed, germline alterations in NF2 are more likely to be nonsense or frameshift mutations (166,169) while missense mutations are more frequently found in sporadic cancers when compared with patients with neurofibromatosis type 2 (166,169). However, this explanation is not complete because there is overlap between the germline NF2 mutations and those found in NF2-bearing sporadic cancers (166,169). Further investigation is required to understand the mechanisms of NF2 mutations and tumorigenesis, which may lead to the identification of novel approaches targeting NF2 in both patients with neurofibromatosis type 2 and in sporadic cancers with this mutation. Interestingly, Subbiah et al. (170) reported a patient with neurofibromatosis type 2 and multiple neoplasms (meningioma, ependymoma and schwannomas) that showed regression when treated with an mTOR inhibitor-containing regimen (and it is known that NF2 mutations activate the mTOR pathway). Trials using targeted therapies in patients with neurofibromatosis type 2 are ongoing.

Implications for Prevention Strategies

Despite the genetic driver complexities in premalignancy, the first precision medicine prevention trial was reported recently in high-risk (presence of genetic driver) oral premalignancy (171), beginning a new era of molecular selection trial designs in cancer prevention (172). Remarkable progress in genome-wide sequencing, big-data analytics, liquid-biopsy technologies, and
deep understanding of the inflammatory tumor microenvironment are decoding the molecular and cellular factors that influence the development of cancer, transforming cancer prevention (173).

Of interest in this regard, activating EGFR mutations, present in about 20% of lung adenocarcinoma (and associated with dramatic responses to EGFR inhibitors), have been reported to be a very early genetic driver in the development of this subset of lung cancer, detected in 43% of histologically normal epithelium surrounding (within and adjacent to) EGFR-mutant lung cancer (174). No mutation was seen in normal lung tissues when lung adenocarcinoma did not harbor EGFR mutation. Preclinical models with mice carrying doxycycline-inducible, lung-specific, mutant EGFR transgenes (L858R or L747–S752 deletion mutation) showed that the EGFR mutation is sufficient for lung cancer development and necessary for tumor maintenance (withdrawal of doxycycline led to tumor regression) (175).

Also of relevance to prevention, ultra-deep sequencing of 74 cancer genes in 234 biopsies of sun-exposed eyelid epidermis revealed two to six mutations per megabase per cell, similar to that seen in many malignancies (176). The most common mutations were in the NOTCH1/2 and FAT1 genes. NOTCH1 driver mutations have also been detected in oral premalignancy (177). Cyclin D1 amplification was one of the first genetic drivers identified in upper aerodigestive tract with severe dysplasia, and Izzo et al. showed that targeting cyclin D1 with retinoid-induced ubiquitinization (proteolysis) reduced genomic instability and cancer rates (178). Data from lung squamous cell carcinoma and recently in lung squamous premalignancy identified 3q26.3–3q29 (including SOX2) amplification/overexpression as a genetic driver in this setting (179). Prevention strategies in high-risk lesions targeting genetic drivers (eg, dysplastic nevi in FAMMM, PI3K pathway in lung dysplasia, HER2 in DCIS) are underway or being planned (as described earlier in this paper).

Recently, driver mutations including BRAF have been detected in circulating cell-free DNA (obtained from blood samples designated liquid biopsies) in patients with lung premalignancy (atypical adenomatous hyperplasia [AAH]) (180). Similarly, in patients with congenital melanocytic nevi, circulating cell-free, DNA-derived BRAF V600E mutations and circulating nevus cells were detected (181). The extent to which such observations might complicate the application of liquid biopsies for prevention or early detection of melanoma or other malignancies, and any potential application of BRAF inhibitors in prevention, remains to be determined.

Although the focus of this paper is on solid tumors, somatic and germline driver mutations have also been well described in some hematologic disorders (eg, germline ETv6 mutations in familial thrombocythemia and diverse hematologic malignancies (182); and somatic JAK2 mutations in myeloproliferative disorders (183)). In some cases, genetic drivers of blood cancers were detected from whole-exome sequencing of DNA in peripheral blood cells from apparently healthy adults and were associated with future blood cancer risk (184). On the other hand, BCR-ABL transcripts (P210BCR-ABL), a hallmark of chronic myelogenous leukemia (CML; but not generally p190BCR-ABL (185,186), are found in the blood of healthy individuals in about 10% to 30% of cases (187–189). Despite this fact, monitoring BCR-ABL transcript levels has been successfully utilized to assess the treatment response from tyrosine kinase inhibitors in patients with CML (190).

Overall, these observations indicate that classic genomic oncogenic drivers can be found in premalignancy and in normal individuals. In some cases, their presence foretells a predisposition to malignancy while in others it may not. The key to successful prevention strategies will therefore be a deep interrogation of the implications of genomic drivers in different contexts (191).

Conclusions

All of the genomic alterations (eg, BRAF, K+, N+, and H-RAS, GNAQ, EGFR, HER2, FGFR3, PIK3CA, ALK, TP53, CDKN2A, NF1, and NF2) discussed herein have been associated with tumor development and implicated as driver alterations (22,89,93,116,135). However, these driver alterations are also variably found in benign and premalignant conditions, occasionally at even higher frequencies than in their malignant counterparts (Table 1). Many of these abnormalities can also be germline. In some cases, they predispose to high rates of malignancy while in other cases they do not.

Overall, several important observations emerge. First, despite classical theories of clonal selection of driver alterations in the progression from benign lesions to premalignancy to malignancy, there appear to be important exceptions. These include BRAF V600E mutations, which are paradoxically more common in benign melanocytic nevi (~80% mutation rate) than in dysplastic nevi (~60%) or melanoma (~40–45%) (13,22–25,37,38); HER2, which is more frequently overexpressed in patients with ductal carcinoma in situ (~27–56% of cases) when compared with its malignant counterpart, invasive breast cancer (~11–20%) (8,12,18,85,86); and FGFR3 mutations, which decrease in frequency with grade of bladder cancer (low-grade tumors, ~61% of cases; high-grade, ~11%) (94,101) (Table 1). In each of these examples, the importance of the alterations to the tumor is beyond doubt, as demonstrated by responses to cognate therapies and other experimental evidence. The presence of these “drivers” at higher rates in benign or premalignant lesions and, in the case of benign melanocytic nevi, their frequent failure to regress with BRAF inhibitor therapy suggest that there is an endogenous inhibitor and/or one or more oncogenic cofactor alterations that are required for malignant evolution to occur (42,43,47,49,50,59,60).

A second observation that becomes apparent is that tissue context may be important in the propensity of a “driver” alteration to produce a cancer. For instance, TP53 mutations are amongst the most common underlying tumor suppressor gene alterations across tumor types. Yet, they can also be found in the synovium of patients with rheumatoid arthritis and, in that tissue context, are not associated with malignancy (Table 1) (137,138). Similarly, identical FGFR3-activating mutations that are seen in solid tumors are also found in benign conditions, such as seborrheic keratoses, with no malignant potential (102–105).

A third pertinent observation relates to the presence of some of these driver aberrations in germline form. In some cases, such as Li-Fraumeni syndrome, whose hallmark is germline TP53 mutations (140), affected individuals are clearly predisposed to a variety of malignancies. However, in other cases, such as dwarfism syndromes whose germline FGFR3 alterations are identical to those found to be drivers in their somatic form, there is no documented increase in cancer in individuals harboring the germline abnormality (112). These findings suggest that the oncogenic potential of certain genomic drivers may also be developmental context-dependent, with early compensatory mechanisms perhaps moderating the oncogenic potential of the alteration.
In summary, the observation of presumed genomic drivers at high frequency in some benign and premalignant lesions, as well as in germline conditions without an increased incidence of cancer, suggests that the functional impact of a gene alteration may be context dependent, with the attendant genomic landscape as well as the tissue and developmental setting being germane. There are several clinically important questions that will need to be addressed. If certain genomic drivers are seen at similar (or greater) frequencies in benign conditions compared with malignant diseases, are we therapeutically targeting the right genetic driver? Are there underlying intact tumor suppressors in benign conditions that prevent driver mutations from promoting tumorigenesis? Can we reliably use genomic alterations as a screening tool for early detection of cancer or as a marker for prevention or treatment response such as with liquid biopsies? Finally, the role of certain driver mutations in tumor initiation and premalignancy, rather than progression and malignancy, merits exploration.

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