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Molecular pathways involved in hair follicle tumor formation: all about mammalian target of rapamycin?

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Abstract: Hair follicle tumors show a broad range of phenotypic variability and diverse histopathological characteristics. To date, different genes and signalling cascades have been implicated in the development and growth of these tumors including the sonic hedgehog, nuclear factor kappa-B and wingless pathway. While the former three have received ample attention, little is known about the possible role of mammalian target of rapamycin (mTOR) in trichofollicular tumorigenesis. Here, we delineate how mTOR can link the various signalling pathways, thereby proposing a unifying model for hair follicle tumor formation.

Key words: hair follicle – hair follicle tumors – mTOR – NF-κB – sonic hedgehog – WNT

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Introduction

The human hair follicle can be a host to several types of benign or malignant tumor. Precise figures are not available, but we estimate that if basal cell carcinoma is not included, about 10% of all skin tumors diagnosed in our clinic are of hair follicle origin. Historically, confusion arises in distinguishing different hair follicle tumor types (Table S1). Their histological appearance is responsible for the conundrum because several of these tumors share common histological characteristics, as previously noted (1,2). Increasingly, however, molecular genetic studies suggest that tumor types once believed to be distinct are phenotypic variations of a single genetic defect. While it is tempting to speculate that alterations at the cellular and genetic level occurring after the primary insult can lead to different growth patterns, there is currently little or no evidence that multiple mutational events occur in benign tumors. Rather, we think that the broad variety in phenotypic and histopathological presentation may reflect local tissue conditions, such as the availability of oxygen and nutrients. Thus, we propose that hair follicle tumors be subdivided according to their underlying molecular defect. This is no longer a strictly nosological exercise because knowledge of

Abbreviations: AKT, V-akt murine thymoma viral oncogene homolog 1 (aka protein kinase B); AMPK, cyclic AMP-activated kinase; APC, adenomatous polyposis coli; BCC, basal cell carcinoma; BHD, Birt–Hogg–Dubé syndrome; BSS, Brooke–Spiegler syndrome; CBP, CREB-binding protein; COS2, costal2; CREB, cAMP response element binding protein; CYLD, cylindromatosis; FGF, fibroblast growth factor; FH, fumarate hydratase; FLCN, folliculin; FZD, frizzled; GLI, glioma transcription factor; GSK3β, glycogen synthetase kinase 3; HIF, hypoxia inducible factor; LEF, lymphoid enhancer factor; LRP, low density lipoprotein receptor-related protein; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa-B; PI3K, phosphatidylinositol-3-kinase; PKA, protein kinase A; PRKAR1a, protein kinase, cAMP dependent, regulatory, type 1a; PTCH, patched; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PTPN11, protein tyrosine phosphatase; S6K, ribosomal protein S6 kinase; SDH, succinate dehydrogenase; SHH, sonic hedgehog; SMAD, suppressor of mothers against decapentaplegic; SOS1, son of sevenless 1; STK11, serine/threonine kinase 11; SUFU, suppressor of fused; TCA, tricarboxylic acid; VHL, Von Hippel–Lindau; VEGF, vascular endothelial growth factor; TRAF2, tumor necrosis factor receptor associated factor-2; VHL, Von Hippel–Lindau; TGFβ, transforming growth factor beta; TSC, tuberous sclerosis complex; WNT, wingless type MMTV integration site.

the molecular pathways involved in the development and growth of specific tumors is starting to have consequences for treatment and follow-up. Recently, exciting developments in molecular oncology suggested that cellular regulation of growth by the mammalian target of rapamycin (mTOR) pathway may be a central theme in tumorigenesis. Here, we discuss the most frequent hair follicle tumors with a known genetic basis and suggest that mTOR can link several signalling networks, thereby providing a unifying concept that may facilitate the development of novel treatment modalities.

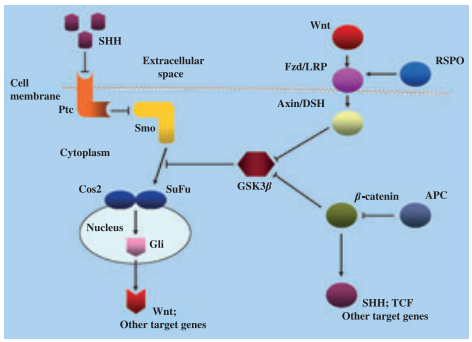

Figure 1. The WNT and SHH signal networks (simplified representation).

The protagonists

Basal cell carcinoma – sonic hedgehog

Basal cell carcinoma (BCC) is the most common malignancy in the Caucasian population (3). Its cell of origin is still a mystery, but it is now generally accepted that BCC is a hair follicle tumor, because several mouse models, such as the *Patched1 +/−*, strongly support a hair follicle origin (4,5). Histology is usually fairly stereotypical, showing basophilic cells, which are classically arranged in more or less globular clusters with palisading cells on their periphery. Several growth patterns exist and these are likely to reflect local intercellular adhesion characteristics most probably mediated by β-catenin (6). However, they do not reflect fundamental biological differences. Sometimes, it is difficult to differentiate BCC from trichoepithelioma (TE) and one might therefore think that in fact, BCC and TE are likely to have a common origin.

Recent data show that virtually all BCCs are caused by mutations affecting components of the sonic hedgehog (SHH) pathway (7,8). About 80% of BCCs have mutations in the *PTCH1* gene coding for Patched1, a 12-pass transmembrane protein that serves as a receptor for SHH. The remaining 20% harbour mutations in either *SMOH* (coding for the Smoothened protein) or in *SU(FU)* (coding for the protein Suppressor of Fused) (7). SHH is not only involved in embryonic hair follicle development but also, later in life, in initiation of the hair anagen phase and does so by regulating the transcription of downstream genes, several of which are known to be involved in hair follicle growth, such as BMP4, Noggin and the wingless type MMTV integration site (WNT) signalling system (9).

WNT signals in turn are transduced by a complex cascade involving glycogen synthetase-kinase 3β (GSK3β, Fig. 1). In the absence of WNT signalling, a cytoplasmic degradation complex, consisting of at least Axin, adenomatous polyposis coli (APC) protein and GSK3β leads to the phosphorylation of APC, β-catenin and Axin by GSK3 (10). This promotes ubiquitination of β-catenin and its subsequent proteasomal degradation (11). Under the influence of a WNT signal, dishevelled proteins bind to the degradation complex and reduce the function of GSK3β (12). Thus, β-catenin is not degraded, translocates to the nucleus and associates with transcription control factor/LEF1. This transcription factor complex is involved in the initiation of the anagen phase (13). Moreover, a LEF1 knockout mouse lacks hair (and other structures that depend on epithelial-mesenchymal interactions) (14,15), proving that this transcription factor is needed for the development of hair growth. Interestingly, activating mutations in β-catenin have been found in pilomatricoma (16), showing that β-catenin is strongly involved in hair growth. Thus, activation of the SHH pathway sets in motion a complex downstream sequence of events that by themselves and in concert can lead to uncontrolled cell growth.

From the above, one might erroneously deduce that the molecular events governing the growth of BCC are so complicated that simple interventions will not suffice to inhibit tumor expansion. Interestingly, however, solely inhibiting SMOH by means of cyclopamine (17) can stop BCC growth (18), implicating the SHH pathway as the major determinant of this tumor.

Cylindroma, trichoepithelioma and spiradenoma – NF-κB

As it happens, β-catenin also interacts with the nuclear factor kappa-B (NF-κB) pathway, connecting the latter with SHH signalling. Increasing evidence suggests that NF-κB activation can regulate activity of genes downstream of β-catenin (19). Likewise, GSK3β has emerged as an indirect regulator of NF-κB activity (20). Thus, it may not come as a surprise that defects in NF-κB signalling (outlined in Fig. 2) can also give rise to hair follicle tumors. NF-κB is a multi-protein complex that can be inhibited by the so-called IkB proteins. These in turn are regulated by IkB kinases (IKK); phosphorylation of IkB proteins by their kinases targets them for ubiquitination and subsequent destruction (21). As a consequence, the NF-κB complex is released. One of the IKK is NEMO, mutations in which cause incontinentia pigmenti and related ectodermal dysplasias

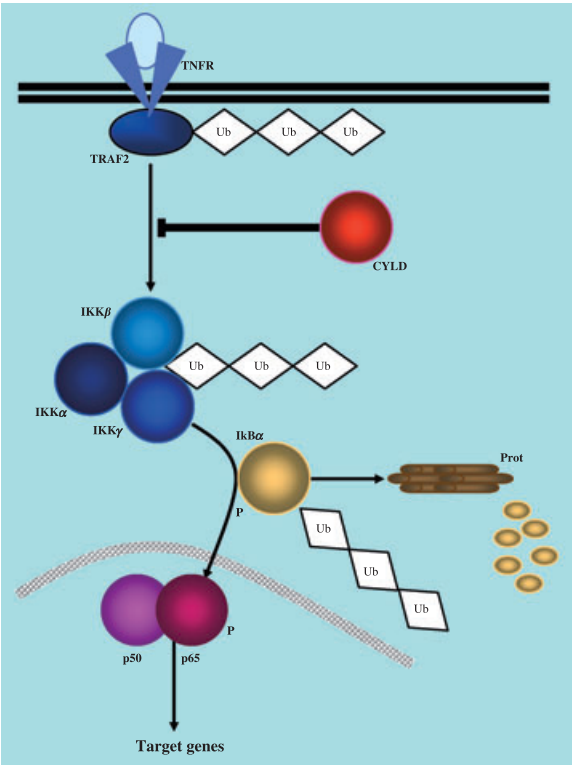

Figure 2. The NF-κB pathway. Ub, ubiquitin; Prot, proteasome.

showing that NF-κB is required for the development of epidermal appendages (22), (23). In 2003, another regulatory element was uncovered when cylindromatosis (CYLD), the product of the cylindromatosis gene, turned out to regulate NEMO (24). CYLD is an ubiquitin hydrolase that targets the auto-ubiquitinating tumor necrosis factor receptor associated factor-2 (TRAF2). Ubiquitinated TRAF2 can activate IKK, which in turn phosphorylates the NF-κB inhibitor IkBα, triggering nuclear translocation of active NF-κB and thus, in the absence of CYLD, leads to an uncontrolled NF-κB activity (25). NF-κB can either induce apoptosis or rather its opposite, cell growth and survival (26). The choice depends on the nature of the signals offered to the receptors upstream of NF-κB. In the hair follicle, NF-κB has an anti-apoptotic effect (27). This mechanism might underlie familial cylindromatosis, also known as Brooke–Spiegler syndrome (BSS), which is caused by mutations in the *CYLD* gene (28). On one hand, the disorder is characterized by benign tumors originating from hair follicle epithelium, called cylindroma (29). On the other hand, we now know that TEs and spiradenomas, a benign tumor originating from sweat duct epithelium (30), can also be found in the context of this disease, suggesting the possibility that cylindroma, TEs and spiradenomas are in fact tumors with an identical molecular background. Indeed, these tumors can manifest simultaneously in one patient (31) and molecular studies have shown that a single *CYLD* mutation can give rise to both cylindroma and multiple TEs (32,33). The histological appearance of the tumors encountered in BSS reflects unbridled growth of the folliculo-sebaceus-apocrine epithelium and will depend on the cell of origin. The initial molecular event is apparently identical in all neoplasms found in this syndrome and this, in our view, justifies categorizing them under one heading. Hence, folliculo-apocrinoma might be a suitable term to define these tumors.

Interestingly, we have also observed occurrence of a BCC in a patient with classical BSS (MvS, unpublished data). One possible explanation might be that it was a TE originally that went on to acquire a SHH pathway mutation under the influence of CYLD, thus transforming into a BCC. An intriguing alternative explanation might be that BCC and cylindroma represent different extremes of a continuum of hair follicle tumors caused by defects in the NF-κB pathway. As the SHH and NF-κB pathways interact, we might expect some BCCs to harbour mutations in the *CYLD* gene. These may be found for example, in individuals who develop multiple BCCs in the absence of excessive solar damage or *PTCH* mutations, a rather common patient category. Such a finding would have important therapeutic implications because there are indications that the effects of CYLD inactivation can be partly overcome by the administration of salicylic acid (24).

Trichilemmoma, fibrofolliculoma, leiomyoma and angiofibroma - united by mTOR

The mTOR signalling network is emerging as one of Nature's most pivotal molecular pathways. If it is to grow, each cell needs to make sure that it has sufficient nutrients and oxygen available. To evaluate its surroundings and nutrient state, the cell has an intricate network of sensor systems. We know about this network because of the severe consequences that absence of one of its components can have. Oxygen availability is translated into a cellular response by an intricate series of events. When sufficient oxygen is available, the Von Hippel–Lindau (VHL) protein, an E3 ubiquitin ligase, marks the protein complex hypoxia-inducible factor (HIF) for proteasomal degradation by ubiquitinating its components (34,35). This process depends on proline hydroxylation of certain residues of HIF-1alpha, one of the HIF subunits. Proline hydroxylation is regulated by oxygen tension (34). In hypoxic conditions, the VHL protein cannot ubiquitinate HIF, which is then stabilized to regulate the expression of several genes whose protein products address the needs of the oxygen-starved cell. These proteins include vascular endothelial growth factor (VEGF), various glucose transporters such as GLUT1 and glycolytic enzymes such as CA9 (36). Thus, it becomes clear why individuals with VHL syndrome develop vascular

tumors in their brains, eyes and kidneys – their VHL protein no longer regulates HIF. Of course, mere availability of oxygen is not the only prerequisite for cell viability. The cell also needs to gauge its surroundings for the presence of nutrients and growth factors that tell it to start dividing, synthesize proteins or rather exit the cell cycle. In this context, 5′AMP activated protein kinase (AMPK) functions as a 'food sensor'. When cellular AMP levels rise, AMPK induces the tuberous sclerosis complex (TSC1 and TSC2, or hamartin/tuberin) by phosphorylation (37). The TSC1/2 complex serves as a GTPase activating protein, or GAP, to the GTPase Rheb. TSC1/2 catalyses the conversion of GTP-loaded Rheb to its GDP loaded form (38), which subsequently inactivates the mTOR complexes via an as yet unknown mechanism (39). Two distinct mTOR complexes with different functionalities are currently known (TORC1 and TORC2), but here we will only focus on TORC1.

TORC1 exerts profound effects on cell size. Experiments in Drosophila have shown that the mTOR pathway is involved in global growth regulation (40). Indeed, mTOR is so important that it even regulates nutrient uptake in the brain (41). The protein LKB1, which is sensitive to low ATP, activates TSC2 when phosphorylated by AMPK (42). In the presence of adequate food supplies, AMPK becomes inactive. As a consequence, the TSC1/2 complex is then also inactivated and mTOR is released from inhibition. This intricate cascade of events, outlined in Fig. 3, also integrates growth factor signalling. The canonical route begins with PI3K (phosphatidylinositol-3-kinase), responding to insulin or insulin-like growth factor (IGF) signals. The signal is then transmitted through PDK1 and Akt/PKB (43). The latter is a kinase that transduces the growth signal to TSC2 and inactivates it, again releasing mTOR from inhibition (44). Extensive cross-talk ensures tight integration of all signals. For instance, the TSC1/2 complex can inhibit HIF, in addition to mTOR (45). The latter is inhibited by HIF, which makes sense because a cell that is hypoxic should make sure that it does not squander its resources (46). In this way, the cell can 'decide' when to grow. The important regulatory role of mTOR in hair follicle and hair follicle-derived tumor growth is reflected by the phenotypes associated with dysfunction of one of its components.

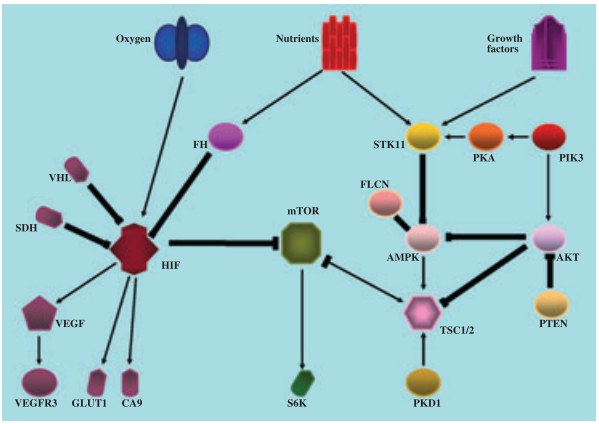

Figure 3. mTOR signalling. Note the central position of HIF.

Several components of the skin are hypoxic to varying degrees, particularly the hair follicle (47). Thus, hypoxia responses mediated by HIF need to be kept strictly in check. In this context, patients with familial leiomyomatosis develop painful smooth muscle tumors, piloleiomyomata, which arise from the musculus arrector pili surrounding the hair follicle. Additionally, uterine leiomyomata and kidney malignancies are frequently observed (48). The disease is caused by heterozygous mutations in the *FH* gene coding for fumarate hydratase (FH), a component of the Krebs tricarboxylic acid (TCA) cycle (49). It may not be immediately obvious how leiomyomatosis relates to mTOR, but the results of recent molecular research have uncovered a beautiful connection. FH deficiency results in intracellular accumulation of fumarate, which stabilizes HIF (50). Hypoxia arrests the TCA cycle, resulting in fumarate accumulation, fumaric acid being one of the intermediates of this metabolic cascade. Further support for this notion is provided by the phenotype of succinate dehydrogenase (SDH) deficiency, in which succinate accumulates in mitochondria and is transported from there to the cytoplasm. There, it inhibits prolyl hydroxylases, which under normoxic conditions enable the VHL protein to target HIF (51). Thus, it would seem that HIF stabilization is a central theme here, although it is not known why FH deficiency gives rise to piloleiomyomata and SDH deficiency to paragangliomas. In this context, other as yet unknown genetic interactions will certainly play an important role.

Another striking example for the involvement of mTOR in the formation of benign skin tumors is tuberous sclerosis (TSC). The hallmark lesions of this disease are angiofibromas, also incorrectly known as adenoma sebaceum. Angiofibroma is a vascular tumor that probably originates from perifollicular fibrous tissue rather than from the hair follicle epithelium (52). In TSC, expression of VEGF and HIF is inappropriately upregulated, while mTOR is also active (45,53). It is not clear why fibrous tissue proliferation occurs in TSC. In other disorders of mTOR signalling, the hair follicle proper seems to be preferentially involved. For example, patients with Birt–Hogg–Dubé (BHD) syndrome, a disease that resembles TSC in many respects, almost invariably have facial hair follicle tumors, fibrofolliculomata. They can also manifest lung cysts and are at increased risk of developing kidney cancer (54). Fibrofolliculoma probably originates from the outer

root sheath. It is characterized by the presence of anastomizing epithelial strands surrounding a distorted hair follicle (55). However, a prominent fibrous component can often be found (MvS, unpublished data) and in this respect, it closely resembles an angiofibroma. Interestingly, angiofibromata have also been described in BHD (56). This observation suggests that the BHD syndrome protein, folliculin (FLCN), may talk with the TSC1/2 complex. In line with this notion, it was recently demonstrated that FLCN is involved in the function of AMPK that regulates mTOR activity, although the nature of this interaction is not yet clear (57). Therefore, it would be of interest to examine angiofibromata for the presence of epithelial components. We would not be surprised if it eventually turned out not to be too different from a fibrofolliculoma.

There is no convincing explanation yet for the appearance of connective tissue tumors around hair follicles in mTOR pathway diseases, but recent data are beginning to provide an explanation. mTOR seems to be directly involved in modulating suppressor of mothers against decapentaplegic (SMAD) activity. For instance, rapamycin can induce SMAD activity in prostate cancer cells (58). SMADs are responsible for transforming growth factor beta (TGFβ) signal transduction in several cell types. TGFβ, among many other functions, mediates collagen production in fibroblasts (59). Thus, dysregulation of mTOR can interfere with TGFβ signaling, which might explain the fibrosing nature of the hair follicle tumors found in mTOR pathway diseases.

United by mTOR?

Several signalling pathways are involved in the development and progression of seemingly different kinds of hair follicle tumors, including the SHH cascade, the WNT system, the NFkB axis and the HIF/mTOR pathway. At first sight, these four pathways could conceivably exert their effects on tumor formation independent of one another. However, upon taking a closer look, specific interactions between these pathways unify them on a molecular avenue that may be the path toward defining a common genetic basis for hair follicle tumors.

It is beyond doubt that mTOR regulates HIF1α expression and its transcriptional activity (60). Through transcriptional regulation of its target genes HIF1α plays a crucial role in tumor cell adaptation to the hypoxic microenvironment. These genes in turn govern a variety of biochemical and cell biological processes, including angiogenesis, apoptosis and glucose metabolism. So, why do disorders of the mTOR pathway give rise to hair follicle tumors? As HIFs mediate transcriptional responses to localized hypoxia in normal tissues such as the hair follicle, dysregulation of such signals can promote tumor formation and progression by altering cellular metabolism and stimulating angiogenesis. However, hypoxia of the hair follicle and dysregulation of mTOR and HIF alone certainly do not suffice to cause tumor growth. Rather, a connection between the mTOR pathway and SHH/WNT signalling may be required. Recent data show that indeed there is a link, which would make biological sense (56,57). After all, the SHH pathway initiates hair growth and it will only do so if sufficient nutrients and oxygen are available. Thus, it should be able to crosscheck with the nutrient-sensing systems.

As noted above, SHH and WNT cross-talk through GSK3β. Inoki et al. (61) recently found that GSK3β can phosphorylate TSC2. WNT signalling inhibits GSK3β and thereby stimulates mTOR signalling. Thus, TSC2 integrates SHH and WNT signals with metabolic status and other growth factor signals to 'decide' whether the cell should be allowed to grow. With this knowledge, we can now begin to understand why dysregulation of mTOR can drive hair follicle tumor formation. Feedback loops are the rule rather than the exception in nature and we might certainly expect one or more here. As a matter of fact, it was recently reported that the mTOR target S6K can regulate GSK3β (62). In cells lacking TSC1 or TSC2, GSK3β was constitutively phosphorylated, leading to a decrease in its activity, thereby mimicking the presence of a WNT signal. Deregulation of mTOR results in increased S6K phosphorylation and will thus stimulate cell growth through WNT signalling.

From the aforesaid, we would like to deduce a tentative integrative model linking the various signalling pathways involved in hair follicle tumor formation as depicted in Fig. 4. Without doubt, several other pathways that are outside the scope of this review may also be crucial for these processes.

Future outlook

Historically, hair follicle tumors were managed with the scalpel and more recently, with the laser. Although results may be satisfactory to the patient, the physician should be happy with nothing less than complete cure or prevention. Once the basic mechanisms of tumor formation are elucidated, this ideal comes within reach and the most eminent interest must then be to develop effective therapeutic strategies based on these pathomechanistic insights. Whenever mTOR is the driving force in disease pathogenesis as, e.g. in the development and progression of hair follicle tumors, treatment with rapamycin or its analogue (so-called 'rapalogs') such as everolimus or temsirolimus (63) should be considered. While rapamycin has many side-effects that might render its long-term use unsafe, the newer rapalogs have a more favourable safety profile. Preliminary trials in

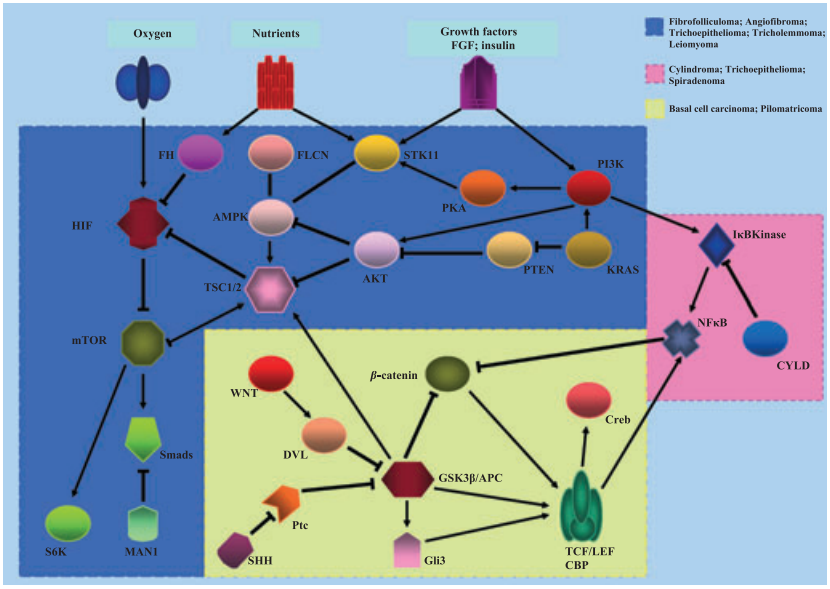

Figure 4. An attempt at integration of multiple signalling networks known to be relevant to hair follicle tumor growth. Only direct inputs into the network are shown. Data obtained using the Bioinformatics Harvester (http://www.harvester.embl.edu) and verified using BioSphere software by Genomatix Inc (http://www.genomatix.de). Coloured boxes indicate functional groups involved with particular tumor types, so that: blue, mTOR group; teal, sonic hedgehog group; pink, NF-κB group.

TSC suggest that disease progression may indeed be halted to a certain degree (64). These promising findings should motivate physicians and scientists to consider seriously the treatment of patients with TSC and related conditions with rapalogs. Likewise, salicylic acid for the tumors of BSS (24) and cyclopamine for those with SHH involvement, such as BCC, could be considered for clinical use. The latter is of particular interest for patients with basal cell nevus syndrome.

Regardless of our advances in understanding biological pathways and the attendant possibilities of intervention, the ideal remains to correct genetic defects on the most basic level, that of the mutation. Recent developments suggest that it may be clinically feasible to correct, or rather, circumvent the consequences of nonsense mutations. It was already known for a long time (65) that high doses of aminoglycoside antibiotics can effect transcriptional read-through, a concept that was proven for among others, cystic fibrosis and Duchenne's muscular dystrophy (66). However, toxicity at high doses limits the applicability of aminoglycosides. While local application is possible for a number of skin diseases, several genetic disorders are multisystem diseases, necessitating systemic treatment. Therefore, less toxic alternatives are required. An exciting new compound, called PTC124, promises to change this, effecting read-through of premature stop codons at relatively low and non-toxic doses (67). PTC124 is currently in clinical trials. For patients with diseases caused by dominant negative mutations, new treatments based on RNA interference are on the horizon (68). Thus, we are living in exciting times and can look forward to major changes for the better in the management of hair follicle tumor syndromes.

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References

1 Schulz T, Ebschner U, Hartschuh W. Localized Birt–Hogg–Dube syndrome with prominent perivascular fibromas. Am J Dermatopathol 2001: **23**: 149–153.
2 Pham T T, Selim M A, Burchette J L, Jr., Madden J, Turner J, Herman C. CD10 expression in trichoepithelioma and basal cell carcinoma. J Cutan Pathol 2006: **33**: 123–128.
3 Tilli C, Van Steensel M, Krekels G, Neumann H, Ramaekers F. Molecular aetiology and pathogenesis of basal cell carcinoma. Br J Dermatol 2005: **152**: 1108–1124.
4 Corcoran R B, Scott M P. A mouse model for medulloblastoma and basal cell nevus syndrome. J Neurooncol 2001: **53**: 307–318.
5 Oro A E, Higgins K. Hair cycle regulation of Hedgehog signal reception. Dev Biol 2003: **255**: 238–248.
6 Saldanha G, Fletcher A, Slater D N. Basal cell carcinoma: a dermatopathological and molecular biological update. Br J Dermatol 2003: **148**: 195–202.
7 Reifenberger J, Wolter M, Knobbe C B *et al.* Somatic mutations in the PTCH, SMOH, SUFUH and TP53 genes in sporadic basal cell carcinomas. Br J Dermatol 2005: **152**: 43–51.
8 Athar M, Tang X, Lee J L, Kopelovich L, Kim A L. Hedgehog signalling in skin development and cancer. Exp Dermatol 2006: **15**: 667–677.
9 Kelleher F C, Fennelly D, Rafferty M. Common critical pathways in embryogenesis and cancer. Acta Oncol 2006: **45**: 375–388.
10 Hart M J, de los Santos R, Albert I N, Rubinfeld B, Polakis P. Downregulation of beta-catenin by human Axin and its association with the APC tumor suppressor, beta-catenin and GSK3 beta. Curr Biol 1998: **8**: 573–581.
11 Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. beta-catenin is a target for the ubiquitin–proteasome pathway. EMBO J 1997: **16**: 3797–3804.
12 Smalley M J, Sara E, Paterson H *et al.* Interaction of axin and Dvl-2 proteins regulates Dvl-2-stimulated TCF-dependent transcription. EMBO J 1999: **18**: 2823–2835.
13 Jamora C, DasGupta R, Kocieniewski P, Fuchs E. Links between signal transduction, transcription and adhesion in epithelial bud development. Nature 2003: **422**: 317–322.
14 Zhou P, Byrne C, Jacobs J, Fuchs E. Lymphoid enhancer factor 1 directs hair follicle patterning and epithelial cell fate. Genes Dev 1995: **9**: 700–713.
15 van Genderen C, Okamura R M, Farinas I *et al.* Development of several organs that require inductive epithelial–mesenchymal interactions is impaired in LEF-1-deficient mice. Genes Dev 1994: **8**: 2691–2703.
16 Chan E F, Gat U, McNiff J M, Fuchs E. A common human skin tumour is caused by activating mutations in beta-catenin. Nat Genet 1999: **21**: 410–413.

17 Chen J K, Taipale J, Young K E, Maiti T, Beachy P A. Small molecule modulation of smoothened activity. Proc Natl Acad Sci USA 2002: **99**: 14071–14076.
18 Tabs S, Avci O. Induction of the differentiation and apoptosis of tumor cells in vivo with efficiency and selectivity. Eur J Dermatol 2004: **14**: 96–102.
19 Cho M, Gwak J, Park S *et al.* Diclofenac attenuates Wnt/beta-catenin signaling in colon cancer cells by activation of NF-kappaB. FEBS Lett 2005: **579**: 4213–4218.
20 Martin M, Rehani K, Jope R S, Michalek S M. Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. Nat Immunol 2005: **6**: 777–784.
21 Scheidereit C. IkappaB kinase complexes: gateways to NF-kappaB activation and transcription. Oncogene 2006: **25**: 6685–6705.
22 Schmidt-Ullrich R, Tobin D J, Lenhard D, Schneider P, Paus R, Scheidereit C. NF-kappaB transmits Eda A1/EdaR signalling to activate Shh and cyclin D1 expression, and controls post-initiation hair placode down growth. Development 2006: **133**: 1045–1057.
23 Courtois G, Gilmore T D. Mutations in the NF-kappaB signaling pathway: implications for human disease. Oncogene 2006: **25**: 6831–6843.
24 Brummelkamp T, Nijman S, Dirac A, Bernards R. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB. Nature 2003: **424**: 797–801.
25 Reiley W, Zhang M, Wu X, Granger E, Sun S C. Regulation of the deubiquitinating enzyme CYLD by IkappaB kinase gamma-dependent phosphorylation. Mol Cell Biol 2005: **25**: 3886–3895.
26 Gilmore T D. Introduction to NF-kappaB: players, pathways, perspectives. Oncogene 2006: **25**: 6680–6684.
27 Schmidt-Ullrich R, Aebischer T, Hulsken J, Birchmeier W, Klemm U, Scheidereit C. Requirement of NF-kappaB/Rel for the development of hair follicles and other epidermal appendices. Development 2001: **128**: 3843–3853.
28 Bignell G R, Warren W, Seal S *et al.* Identification of the familial cylindromatosis tumour-suppressor gene. Nat Genet 2000: **25**: 160–165.
29 Massoumi R, Podda M, Fassler R, Paus R. Cylindroma as tumor of hair follicle origin. J Invest Dermatol 2006: **126**: 1182–1184.
30 Meybehm M, Fischer H P. Spiradenoma and dermal cylindroma: comparative immunohistochemical analysis and histogenetic considerations. Am J Dermatopathol 1997: **19**: 154–161.
31 Uede K, Yamamoto Y, Furukawa F. Brooke–Spiegler syndrome associated with cylindroma, trichoepithelioma, spiradenoma, and syringoma. J Dermatol 2004: **31**: 32–38.
32 Poblete Gutierrez P, Eggermann T, Holler D *et al.* Phenotype diversity in familial cylindromatosis: a frameshift mutation in the tumor suppressor gene CYLD underlies different tumors of skin appendages. J Invest Dermatol 2002: **119**: 527–531.
33 Zhang G, Huang Y, Yan K *et al.* Diverse phenotype of Brooke–Spiegler syndrome associated with a nonsense mutation in the CYLD tumor suppressor gene. Exp Dermatol 2006: **15**: 966–970.
34 Jaakkola P, Mole D R, Tian Y M *et al.* Targeting of HIF-alpha to the von Hippel–Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 2001: **292**: 468–472.
35 Ohh M, Park C W, Ivan M *et al.* Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel–Lindau protein. Nat Cell Biol 2000: **2**: 423–427.
36 Zelzer E, Levy Y, Kahana C, Shilo B Z, Rubinstein M, Cohen B. Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1alpha/ARNT. EMBO J 1998: **17**: 5085–5094.
37 Kwiatkowski D J, Manning B D. Tuberous sclerosis: a GAP at the crossroads of multiple signaling pathways. Hum Mol Genet 2005: **14** Spec No. 2: R251–R258.
38 Inoki K, Li Y, Xu T, Guan K L. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev 2003: **17**: 1829–1834.
39 Garami A, Zwartkruis F J, Nobukuni T *et al.* Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2. Mol Cell 2003: **11**: 1457–1466.
40 Zhang H, Stallock J P, Ng J C, Reinhard C, Neufeld T P. Regulation of cellular growth by the Drosophila target of rapamycin dTOR. Genes Dev 2000: **14**: 2712–2724.
41 Cota D, Proulx K, Smith K A *et al.* Hypothalamic mTOR signaling regulates food intake. Science 2006: **312**: 927–930.
42 Corradetti M N, Inoki K, Bardeesy N, DePinho R A, Guan K L. Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz–Jeghers syndrome. Genes Dev 2004: **18**: 1533–1538.
43 Paradis S, Ailion M, Toker A, Thomas J H, Ruvkun G. A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in Caenorhabditis elegans. Genes Dev 1999: **13**: 1438–1452.
44 Inoki K, Li Y, Zhu T, Wu J, Guan K L. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol 2002: **4**: 648–657.
45 Brugarolas J B, Vazquez F, Reddy A, Sellers W R, Kaelin W G, Jr. TSC2 regulates VEGF through mTOR-dependent and -independent pathways. Cancer Cell 2003: **4**: 147–158.
46 Brugarolas J, Lei K, Hurley R L *et al.* Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. Genes Dev 2004: **18**: 2893–2904.
47 Evans S M, Schrlau A E, Chalian A A, Zhang P, Koch C J. Oxygen levels in normal and previously irradiated human skin as assessed by EF5 binding. J Invest Dermatol 2006: **126**: 2596–2606.
48 Launonen V, Vierimaa O, Kiuru M *et al.* Inherited susceptibility to uterine leiomyomas and renal cell cancer. Proc Natl Acad Sci USA 2001: **98**: 3387–3392.
49 Badeloe S, van Geel M, van Steensel M A *et al.* Diffuse and segmental variants of cutaneous leiomyomatosis: novel mutations in the fumarate hydratase gene and review of the literature. Exp Dermatol 2006: **15**: 735–741.
50 Isaacs J S, Jung Y J, Mole D R *et al.* HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. Cancer Cell 2005: **8**: 143–153.
51 Selak M A, Armour S M, MacKenzie E D *et al.* Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell 2005: **7**: 77–85.
52 Sanchez N, Wick M, Perry H. Adenoma sebaceum of Pringle: a clinicopathologic review, with a discussion of related pathologic entities. J Cutan Pathol 1981: **8**: 395–403.
53 El-Hashemite N, Walker V, Zhang H, Kwiatkowski D J. Loss of Tsc1 or Tsc2 induces vascular endothelial growth factor production through mammalian target of rapamycin. Cancer Res 2003: **63**: 5173–5177.
54 Khoo S K, Giraud S, Kahnoski K *et al.* Clinical and genetic studies of Birt–Hogg–Dube syndrome. J Med Genet 2002: **39**: 906–912.
55 Collins G L, Somach S, Morgan M B. Histomorphologic and immunophenotypic analysis of fibrofolliculomas and trichodiscomas in Birt–Hogg–Dube syndrome and sporadic disease. J Cutan Pathol 2002: **29**: 529–533.
56 Schaffer J V, Gohara M A, McNiff J M, Aasi S Z, Dvoretzky I. Multiple facial angiofibromas: a cutaneous manifestation of Birt–Hogg–Dube syndrome. J Am Acad Dermatol 2005: **53**: S108–S111.
57 Baba M, Hong S B, Sharma N *et al.* Folliculin encoded by the BHD gene interacts with a binding protein, FNIP1, and AMPK, and is involved in AMPK and mTOR signaling. Proc Natl Acad Sci USA 2006: **103**: 15552–15557.
58 van der Poel H G, Hanrahan C, Zhong H, Simons J W. Rapamycin induces Smad activity in prostate cancer cell lines. Urol Res 2003: **30**: 380–386.
59 Verrecchia F, Mauviel A, Farge D. Transforming growth factor-beta signaling through the Smad proteins: role in systemic sclerosis. Autoimmun Rev 2006: **5**: 563–569.
60 Brugarolas J, Kaelin W. Dysregulation of HIF and VEGF is a unifying feature of the familial hamartoma syndromes. Cancer Cell 2004: **6**: 7–10.
61 Inoki K, Ouyang H, Zhu T *et al.* TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell 2006: **126**: 955–968.
62 Zhang H H, Lipovsky A I, Dibble C C, Sahin M, Manning B D. S6K1 regulates GSK3 under conditions of mTOR-dependent feedback inhibition of Akt. Mol Cell 2006: **24**: 185–197.
63 Dancey J E. Inhibitors of the mammalian target of rapamycin. Expert Opin Investig Drugs 2005: **14**: 313–328.
64 Herry I, Neukirch C, Debray M P, Mignon F, Crestani B. Dramatic effect of sirolimus on renal angiomyolipomas in a patient with tuberous sclerosis complex. Eur J Intern Med 2007: **18**: 76–77.
65 Tai P C, Davis B D. Triphasic concentration effects of gentamicin on activity and misreading in protein synthesis. Biochemistry 1979: **18**: 193–198.
66 Aurino S, Nigro V. Readthrough strategies for stop codons in Duchenne muscular dystrophy. Acta Myol 2006: **25**: 5–12.
67 Welch E M, Barton E R, Zhuo J *et al.* PTC124 targets genetic disorders caused by nonsense mutations. Nature 2007: **447**: 87–91.
68 Grunweller A, Hartmann R K. RNA interference as a gene-specific approach for molecular medicine. Curr Med Chem 2005: **12**: 3143–3161.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Commonly recognized hair follicle tumor types and their molecular background.

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