Chemoprevention and Treatment of Experimental Cowden’s Disease by mTOR Inhibition with Rapamycin

Cristiane H. Squarize, Rogério M. Castilho, and J. Silvio Gutkind
Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, NIH, Bethesda, Maryland

Abstract
Cowden’s disease is an autosomal dominant disorder characterized by the development of multiple mucocutaneous lesions and benign tumors, and enhanced cancer predisposition. Most Cowden’s disease patients harbor inactivating mutations in the PTEN tumor suppressor gene which encodes a lipid phosphatase, PTEN, which restrains the phosphatidylinositol 3-kinase–Akt signaling pathway. We observed that the epithelial-specific deletion of Pten in mice causes multiple hyperproliferative and tumor lesions that strikingly resemble Cowden’s disease. This animal model system provided an opportunity to explore novel therapeutic approaches in Cowden’s disease. Indeed, we show here that rapamycin administration, which inhibits a key downstream target of Akt, mammalian target of rapamycin (mTOR), promotes the rapid regression of advanced mucocutaneous lesions. Furthermore, when administered before disease manifestation, rapamycin can halt the development of Cowden’s disease–like lesions, thereby prolonging animal survival. These findings suggest that mTOR inhibition with rapamycin may represent a suitable therapeutic option for the chemoprevention and treatment of Cowden disease patients and others tumor syndromes that involve defective PTEN function. [Cancer Res 2008;68(17):7066–72]

Introduction
Cowden’s disease is an autosomal dominant disorder characterized by the development of multiple mucocutaneous lesions and benign tumors, and by the predisposition to a variety of malignancies, particularly breast and thyroid cancers (1–6). The Cowden’s disease gene locus has been mapped to the chromosome 10q22-23 in which the PTEN gene is located (5–8). PTEN encodes a lipid phosphatase, PTEN, which is a negative regulator of the phosphatidylinositol 3-kinase–Akt pathway by converting phosphatidylinositol 3,4,5-triphosphate (PIP3) into phosphatidylinositol 4,5-biphosphate (PIP2; refs. 9–11). Underscoring the importance of PTEN as a tumor suppressor gene, germline mutations of PTEN have been linked to several autosomal dominant hamartoma syndromes including Cowden’s disease (OMIM:158350), Bannayan-Riley-Ruvalcaba syndrome (OMIM:153480), and Lhermitte-Duclos syndrome (OMIM:158350), which are all characterized by the presence of benign tumors, known as hamartomas, in multiple organs and an increased susceptibility to developing a variety of malignancies (reviewed in ref. 12).

In the case of Cowden’s disease, PTEN germline mutations are found in 80% of the patients, either in the PTEN coding sequence, in the PTEN promoter, or in its 5′ and 3′ untranslated region resulting in inhibition of translation or a catalytically inactive, immature, or unstable PTEN protein, which may undergo rapid degradation (10, 12). Pten heterozygosity and tissue-specific deletion in mice leads to hyperplastic and dysplastic changes in the prostate, colon, and skin, and to spontaneous tumor development, supporting the notion that PTEN plays a causal role in hamartoma syndromes (13–19). Cells exhibiting decreased PTEN activity are not able to restrain the growth promoting properties of PI3K and its lipid product, PIP3 (10, 11, 20). One of the best studied downstream targets of PI3K is the serine-threonine kinase Akt, which, upon activation by PIP3, promotes cell proliferation and survival by phosphorylating multiple protein targets, thereby controlling cell growth, protein translation, cell metabolism, and program cell death (21, 22).

As Cowden’s disease is characterized by the high incidence epithelial derived tumors, we selectively inactivated a floxed Pten allele in mice by the expression of Cre recombinase under the control of the keratin 14 (K14) promoter, which is expressed in hair follicles, glandular ducts, epidermis, and other epithelial cells (23, 24). Although mice developed normally, and newborn mice did not exhibit any obvious phenotype, Pten deletion in epithelial cells led to the development of multiple dermal lesions and breast and thyroid tumors as age progressed, strikingly resembling Cowden’s disease. This animal model provided an opportunity to explore the efficacy of interfering with the PI3K/Akt pathway as a therapeutic approach in this hamartoma syndrome. Indeed, we show here that the pharmacologic inhibition of one particular downstream target of Akt, mammalian target of rapamycin (mTOR), by the prolonged treatment with rapamycin promoted the rapid regression of advanced Cowden’s disease–like lesions. Furthermore, we found that by initiating the chronic administration of rapamycin before the appearance of disease manifestation in mice, in which Pten has been conditionally deleted, was sufficient to prevent the development of Cowden’s disease–like lesions, thereby prolonging remarkably animal survival. Thus, these findings suggest that the blockade of mTOR with rapamycin and its analogues may represent a suitable therapeutic option for the chemoprevention and treatment of Cowden disease’s patients.

Materials and Methods

Experimental mice. All animal studies were carried out according to NIH approved protocols, in compliance with the Guide for the Care and Use of Laboratory Animals. PtenF/F mice (The Jackson Laboratory; ref. 25) were crossed with K14Cre mice (24) to generate K14Cre PtenF/2 heterozygous mice, which were further crossed with PtenF/F mice to generate K14Cre PtenF/F (homozygous), K14Cre PtenF/2, PtenF/F, and PtenF/2 mice, all in the
same litter. The mice had free access to water and pellet stock diet, with the addition of high-fat supplement, when needed. Genotyping was performed on tail biopsies using a PCR assay with primers P1 (5’ acgaaggaaggttgcagc-3’) and P2 (5’ ggccagttgacaaagatg 3’) for Pten\textsuperscript{fl/fl}, and primers P3 (5’ cacgataacaggtctggttg 3’) and P4 (5’ cattccacagctagtggeact 3’) for K14-Cre.

Rapamycin administration. Rapamycin (LC Laboratories) was reconstituted in absolute ethanol at 10 mg mL\textsuperscript{-1} and stored at −20°C. Rapamycin was diluted in 5.2% Tween 80 (Sigma) and 5.2% polyethylene-glycol (PEG-400; Hampton Research; ref. 26), and injected ip., 1 mg/kg every other day.

Culture of primary keratinocytes and Western blotting. Murine keratinocytes were isolated as described (23), grown in KRB-2 medium (Cambrex). After lyses, protein concentrations were determined and 30 g of proteins were separated on 10% SDS-PAGE, transferred to nitrocellulose membranes, blocked with 5% milk protein, and incubated with primary antibodies Akt, phospho-specific Akt (pAkt\textsuperscript{Ser473} and pAkt\textsuperscript{Thr308}), and S6 (pS6; Cell Signaling Technology), PTEN (Cascade Bioscience), and tubulin (Santa Cruz Biotechnology), as previously described (27). Western blot signals were quantitated by using the NIH Image J software.\textsuperscript{1}

Histology and immunohistochemistry of tissue sections. H&E staining was performed on formalin-fixed and paraffin-embedded 4-m serial sections according to standard procedures. Immunohistochemistry was performed on these paraffin-embedded and frozen tissue sections using antibodies pAkt\textsuperscript{Ser473}, pAkt\textsuperscript{Thr308}, pS6 (Cell Signaling Technology), and proliferating cell nuclear antigen (PCNA; Zymed Laboratories) as described previously (26, 28). Quantitative analysis of the staining intensity was performed by using the NIH Image J software. Procedure periodic acid-Schiff (PAS) staining (Sigma-Aldrich) was performed as described by the manufacturer.

Statistical analysis. Comparison of the Kaplan-Meier survival analysis was performed with the logrank test. Statistical analysis of the staining for PCNA, pAkt\textsuperscript{Ser473}, pAkt\textsuperscript{Thr308}, pS6, and the body weight was performed by ANOVA One-way, followed by the Bonferroni’s multiple comparison tests using GraphPad Prism 4.03 (GraphPad Software).

Results

Excision of Pten recapitulates features of Cowden’s disease. We generated epithelial-specific Pten conditional knockout mice by crossing mice harboring a floxed Pten allele containing two loxP sites (Pten\textsuperscript{floxed}) with mice expressing the Cre recombinase under the control of the K14 promoter (K14-Cre). K14-Cre Pten\textsuperscript{floxed} heterozygous mice were backcrossed with Pten\textsuperscript{floxed} mice, and littermates that inherited some but not all of the above alleles served as controls. K14-Cre Pten\textsuperscript{floxed} mice were undistinguishable from the littermates at birth. However, at ages 6 days, the skin was wrinkled and flaky when compared with heterozygous K14Cre Pten\textsuperscript{+/f+} and control mice, and by ages 11 days, the hair shafts had a disheveled appearance (data not shown). The animals progressively developed multiple hyperproliferative lesions, ranging from mucocutaneous lesions (95%; 41 of 44 mice) to tumor formation (Fig. 1A; Table 1). In particular, multiple papules rapidly developed around the facial orifices such as nose, mouth, and eyes (Fig. 1A, top). Punctuate palmpoplantar keratoses, acral keratosis, especially in the limbs and periauricular, and oral papules were also frequently observed (Fig. 1A, bottom). Histologically, most facial and periauricular lesions had a follicular component with hyperproliferation of the outer root sheath, also evident in the vibrissae (Supplementary Fig. S1A; ear and vibrissae). Papillomatous lesions of the skin presented hyperkeratosis and acanthosis throughout the face and body skin (Supplementary Fig. S1A; skin and head). Acral extremities areas displayed verrucous and hyperkeratotic lesions also seen in the punctuate palmpoplantar keratosis of the paws (Supplementary Fig. S1A; paw).

Deletion of Pten compromises animal survival. Initially, the offspring from mating K14Cre Pten\textsuperscript{+/f+} mice with Pten\textsuperscript{floxed} mice followed the expected Mendelian distribution (Supplementary Fig. S2A). Although rapid death was initially observed in K14Cre

Figure 1. Widespread mucocutaneous lesions and early lethality caused by the conditional deletion of Pten in epithelial cells. A, representative examples of Pten conditional knockout mice, K14Cre Pten\textsuperscript{floxed}, and littermate controls as indicated. Multiple facial papules extensively involving the eyes and nose (top). Punctuate palmpoplantar keratoses of the paws (middle). Facial papules around the mouth, acral keratosis in the limbs, and oral papilloma of the buccal mucosa (bottom). B, survival curves for K14Cre Pten\textsuperscript{floxed} (n = 71), K14Cre Pten\textsuperscript{+/f+} (n = 22), and controls (n = 56; Kaplan-Meier survival analysis, P < 0.0001).

1 http://rsb.info.nih.gov/ij
Pten<sup>F/F</sup> mice in the first few days of life (Fig. 1B; Supplementary Fig. S2A), their survival was much greater than observed when using Cre driven by the K5 promoter (14). Although K14- and K5-driven gene expression are expected to have somehow overlapping tissue specificity (29), K14Cre Pten<sup>F/F</sup> mice did not develop esophageal hyperplasia, which could compromise the survival of K5Cre mice. In addition, we were able to achieve an increase in animal survival of K14Cre Pten<sup>F/F</sup> mice by removing littermate competition and extending the weaning period (Supplementary Fig. S2A). Under these optimized breeding conditions, most animals survived until weaning, and 40% of the mice survived beyond ages 3 months, although the life span of K14Cre Pten<sup>F/F</sup> mice did not exceed 250 days (Fig. 1B; Supplementary Fig. S2A). Decreased survival was also present in heterozygous K14Cre Pten<sup>F/+</sup> mice, which died or had to be sacrificed because of morbidity (Fig. 1B). Nonetheless, the ability to extend the survival of K14Cre Pten<sup>F/F</sup> mice for up to ages 9 months enabled us to explore in detail the nature of the pathologic conditions caused by Pten deletion in the epithelial compartment of mice.

Hyperproliferative and tumoral lesions in K14Cre Pten<sup>F/F</sup> mice. As described above, several cutaneous lesions could be readily observed during examination of K14Cre Pten<sup>F/F</sup> mice. They include the unique presence of trichilemmomas, a pathognomonic sign of Cowden’s Disease (2) that resembles blown-up hair follicles, which often exhibit cylindrical or lobular proliferation and PAS-positive proliferating glycojen-rich clear cells (Fig. 2A, top). Besides numerous skin alterations, Cowden’s disease is characterized by thyroid multinodular goiter, adenomas, and increased risk for thyroid cancer (4, 8). Indeed, homozygous deletion of Pten resulted in alterations in the thyroid gland of K14Cre Pten<sup>F/F</sup> mice (n = 16), including multinodular goiters (56%; 9 of 16 mice analyzed) and thyroid tumors (12%; 2 of 16 mice analyzed) with a follicular aspect

<table>
<thead>
<tr>
<th>Table 1. Manifestations of Cowden’s syndrome</th>
<th>Human</th>
<th>Animal model</th>
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<tbody>
<tr>
<td>Pathognomonic criteria</td>
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<tr>
<td>Muco-cutaneous lesions</td>
<td>90–100%</td>
<td>95% (41/44)</td>
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<td>Facial papillomatous lesions</td>
<td>+</td>
<td>+</td>
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<td>Trichilemmomas</td>
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<td>+</td>
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<tr>
<td>Acral keratoses</td>
<td>+</td>
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<tr>
<td>(ears, limbs, and fingers)</td>
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<tr>
<td>Palmoplantar keratoses</td>
<td>+</td>
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<td>Oral mucosa papillomatosis</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Major criteria</td>
<td></td>
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<tr>
<td>Breast cancer</td>
<td>30–76%</td>
<td>36% (8/22)</td>
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<tr>
<td>Macroecephalia/Lhermitte-Duclos</td>
<td>38%</td>
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<tr>
<td>Disease</td>
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<tr>
<td>Thyroid cancer</td>
<td>3–10%</td>
<td>2% (2/16)</td>
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<tr>
<td>Other lesions and symptoms</td>
<td>44%</td>
<td>—</td>
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<tr>
<td>Intestinal polyps</td>
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<tr>
<td>Genitourinary lesions</td>
<td>5–44%</td>
<td>4.5% (2/44)</td>
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<tr>
<td>Thyroid Abnormalities</td>
<td>50–67%</td>
<td>56% (9/16)</td>
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altersations in the nipples. Multiple mammary gland tumors were also observed in the thoracic and abdominal/inguinal mammary glands of K14Cre Pten<sup>F/F</sup> mice (11%; 4 of 36 mice analyzed; Fig. 2B: Table 1). This was even more remarkable in K14Cre Pten<sup>F/+</sup> mice (36%; 8 of 22 mice analyzed), as their extended survival enabled the development of larger tumoral lesions. In both cases, lesions were characterized by the presence of prominent intraluminal and ductal proliferation (Fig. 2B, b). Histologic features of breast tumors ranged from fibroadenomas (Fig. 2B, c) to adenocarcinomas and ductal carcinomas with dense hyaline collagen or dense fibrosis replacing the intralobular stroma (Fig. 2B, e and f).

Reversion of Cowden’s disease–like lesions by mTOR inhibition. Activation of Akt involves its recruitment to the membrane by binding PIP<sub>3</sub> and the initial phosphorylation of threonine 308 (Thr308) in its activation loop by PDK1 (22, 31). Subsequently, Akt is phosphorylated in serine 473 (Ser473) by one of its downstream targets, the atypical kinase mTOR, thereby increasing its activity (31). mTOR in turn regulates protein...
Figure 3. Rapamycin treatment reverses Cowden’s disease–like lesions and modulates mTOR pathway in K14Cre PtenF/F–derived tumors. A, representative examples of rapamycin-treated mice (n = 4) within 9 d of treatment. Rapamycin profoundly decreases well-established mucocutaneous lesions in the face (top) and paws (bottom) of the same K14Cre PtenF/F mouse. B, representative example of immunohistochemical staining for pAktThr308, pAktSer473, pS6, and PCNA in the epithelium from control and K14Cre PtenF/F mice treated with vehicle or rapamycin. Note the elevated phosphorylation of pS6 and Akt in Ser473, and PCNA in K14Cre PtenF/F mice. Upon treatment, pS6, pAktSer473 levels, and PCNA nuclear staining were notably reduced. pAktThr308, which is present in the nucleus of epithelial cells in K14Cre PtenF/F mice, shifted to the membrane in rapamycin-treated mice. C, representative Western blot analysis of primary culture from mice for molecules whose activity is regulated by PTEN and tubulin, as a loading control, in lysates from primary cultures of keratinocytes isolated from control (c), K14Cre PtenF/F (f/+), and K14Cre PtenF/F (ff) mice (n = 3 independent experiments per genotype). Cells were treated with vehicle or rapamycin, as indicated. PTEN levels were reduced in K14Cre PtenF/+ and almost absent in K14Cre PtenF/F, which presents increased levels of both pAktThr308 and pAktSer473. Rapamycin effectively ablates S6 phosphorylation, normalizes pAktSer473, and increases pAktThr308 in homozygous K14Cre PtenF/F mice when compared with heterozygous and control mice. C, quantitative analysis of pAktSer473, pAktThr308, and pS6, as fold increase of the control mice (C, bottom, white bar).

Rapamycin treatment clearly diminished the activity of mTOR in vivo, as judged by the dramatically reduced immunohistochemical detection of the phosphorylated form of S6, which was elevated in K14Cre PtenF/F mice when compared with littermate controls (Fig. 3B; Supplementary Fig. S2B). The reduced pS6 levels reflected the inhibition of mTOR as part of its complex 1 (mTORC1), the direct target of rapamycin (38), thus representing a suitable marker for monitoring the activity of rapamycin on its target molecule (see refs. 39–41, and references therein). Concomitantly, rapamycin reduced the proliferation of epithelial cells within the skin lesions, as assessed by PCNA staining (Fig. 3B; Supplementary Fig. S2B), which was quite elevated in Pten-deficient mice when compared with controls (P < 0.001). Aligned with the elevated levels of PI3K lipid products in these Pten-deficient mice, there was an extensive accumulation of Akt phosphorylated in its Ser473 site (pAktSer473) in the skin of the vehicle-treated K14Cre PtenF/F animals (Fig. 3B, top; Supplementary Fig. S2B). Prolonged treatment with rapamycin resulted in a decreased phosphorylation of pAktSer473 (Fig. 3B; Supplementary Fig. S2B). This is likely due to the indirect inhibition of the mTOR2 complex (mTORC2), which phosphorylates Akt on its Ser 473 site, which represents a secondary effect of rapamycin previously described to occur in vivo (38). However, there appeared to be an increased level of pAktThr308 after rapamycin treatment, which also shifted from the nucleus in the K14Cre PtenF/F animals to the membrane. These observations suggest that upon mTOR blockade, the activity of Akt may be enhanced, likely due to the absence of a negative feedback mechanism restraining growth factor activation of Akt (38), similar to that reported during recent clinical trials with rapamycin in PTEN-deficient glioblastoma patients (41). Of interest, however, this active Akt may not be able to be released from the membrane in cells that accumulate PIP3 due to the absence of Pten phosphatase activity, as judged by its membrane accumulation (Fig. 3B, middle).

Elevated levels of pS6 and the effect of rapamycin on pAktThr308 were also observed in primary cultures of keratinocytes isolated from K14Cre PtenF/F mice. As expected, Pten levels were reduced in cells isolated from heterozygous K14Cre PtenF/+ mice, and almost absent in keratinocytes from K14Cre PtenF/F homozygous mice.
We observed increased levels of both pAktSer473 and pS6 in K14Cre PtenF/F homozygous mice. Rapamycin treatment lead to a complete ablation of S6 phosphorylation, although it had only a limited effect on pAktSer473 levels, aligned again with the direct effect of rapamycin on the mTORC1 complex after short time treatment. However, rapamycin provoked an increase in pAktThr308 in primary cultures from Pten-deficient mice when compared with those derived from heterozygous and control mice (Fig. 3C). Thus, the effects of rapamycin on signaling events in the epithelial compartment of the skin of K14Cre PtenF/F mice likely represent a primary effect on the targeted cells, rather than resulting from alterations caused by mTOR inhibition in the stroma.

**Rapamycin as a chemoprevention agent for experimental Cowden’s disease.** Over 90% of individuals affected with Cowden’s disease are believed to manifest clinical signs of the disease by the ages 20 years; 99% of the affected individuals develop mucocutaneous lesions by the end of the third decade, although other clinical manifestations may be present; and tumor formation is usually detected at the beginning of the fourth decade (Fig. 4; refs. 1–4). Of interest, as mice age, K14Cre PtenF/F animals acquired a phenotype that closely resembles the progression of Cowden’s disease (Fig. 4; Table 1). Thus, we asked whether rapamycin administration before the appearance of disease manifestation could prevent the development of Cowden’s disease–like lesions in mice already harboring Pten deletions. For these experiments, we administered a clinically relevant low dose of rapamycin (37) starting at ages 5 days, and compared disease progression in the treated animals to their littermate controls (n = 13 per group). There were no statistically significant differences in the body weight when comparing control mice treated with the rapamycin inhibitor and vehicle (Fig. 4C). The K14Cre PtenF/F mice treated with vehicle (20.9 ± 2.8 g) were slightly smaller than their control littermates (28.1 ± 2.9 g) but gained weight similar to control mice upon rapamycin treatment (24.1 ± 2.5 g; Fig. 4C). Furthermore, rapamycin treatment prevented the development of dermatologic lesions, including facial papules, oral papules, acral, and palmar-plantar keratoses, and increased the life span of K14Cre PtenF/F mice (Fig. 4A and B). This reduction in the mortality of K14Cre PtenF/F mice was quite remarkable (vehicle control: median survival, 94 days; rapamycin: median survival, >320 days; P < 0.0001; Fig. 4B), suggesting that mTOR inhibition may represent a suitable chemopreventive strategy to halt Cowden’s disease progression (Fig. 4D).

**Discussion**

Cowden’s disease was named after the first described patient, Rachael Cowden, in 1963 (1). The discovery of PTEN germline

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**Figure 4.** Prolonged treatment with rapamycin prevents the formation of mucocutaneous lesions and prolongs the survival of mice deficient in Pten. Rapamycin or vehicle was administered i.p. starting at ages 5 d to control, K14Cre Pten+/+; and K14Cre PtenF/F (n = 13 for each genotype). A, whereas K14Cre PtenF/F mice formed lesions, K14Cre PtenF/F from the same litter that were treated with rapamycin did not develop dermatologic lesions. Representative mice are depicted. B, survival curve (Kaplan-Meier survival analysis) of animals treated with vehicle and rapamycin. Rapamycin extended the survival of K14Cre PtenF/F and K14Cre PtenF/F mice (logrank test between K14Cre PtenF/F treated with vehicle and rapamycin, P < 0.0001). C, growth curves of animals treated with vehicle and rapamycin (n = 13 mice per genotype). Points, mean; bars, SE. There were no statistically significant differences in the body weight among any of the indicated groups (P > 0.05). D, cartoon depicting the chronological development of Cowden’s disease clinical manifestations during the lifetime in humans harboring inactivating PTEN mutations and deletions, and epithelial Pten-deleted mice. Arrows, initiation of rapamycin treatment for the chemoprevention (red) or therapy (orange) of experimental Cowden’s disease in mice.
somitic mutations in the majority of Cowden’s disease patients have provided an opportunity for the early diagnosis to individuals that are susceptible to the development of this debilitating and cancer-prone syndrome (5–8). Aligned with the key role of PTEN in Cowden’s disease syndrome, we were able to recapitulate most of the pathognomonic lesions characterizing Cowden’s disease, including trichilemmoma, acral keratoses, papillomatous, and mucosal lesions, and even breast and thyroid alterations and tumors by deleting Pten in stratified and ductal epithelial cells using K14Cre mice, which was combined with the optimization of the housing conditions to extend the life span of the affected mice. The availability of this animal model provided a unique opportunity to explore the ability to interfere with PTEN downstream molecules as potential targets for therapeutic intervention in Cowden’s disease.

Reduced PTEN function, and thus elevated PIP3 accumulation, can result in altered activity of a PI3K-depedent signaling network, which ultimately promotes disease progression (21, 22). Although inhibiting PI3K activity would be a good candidate to limit PIP3, and as a result compensate for PTEN reduced activity, currently, PI3K inhibitors may have undesirable side effects that limit their clinical use (42). Among the many biochemical routes regulated by PI3K, the Akt-mTOR pathway has recently emerged as a key component of proliferative pathways activated downstream from PI3K and PTEN (34, 35, 43). In this regard, loss of mTOR is epistatic to Pten loss in drosophila megalogaster (44, 45). Furthermore, the oncogenic activity of Akt seems to be dependent on mTOR, and many sporadic tumors involving PTEN deletions and inactivating mutations are highly sensitive to rapamycin treatment that blocks mTOR (35, 49). Rapamycin (41) and many of its analogues (rapalogs), including CCI-779 (temsirolimus), RAD001 (everolimus), and AP23573, are in clinical trials for a variety of tumor types, and CCI-779 has been recently approved by FDA for the treatment of renal carcinoma patients (50). Collectively, the extensive experience with the use of rapalogs in the clinic and the key role of mTOR in Cowden’s disease progression may provide the rationale for the early clinical evaluation of rapamycin and its analogues as a molecular-targeted chemopreventive strategy for Cowden’s disease and others tumor syndromes that involve defective PTEN function.

Disclosure of Potential Conflicts of Interest

The authors declare that they have no competing financial interests.

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References


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