The lentiginoses: Cutaneous markers of systemic disease and a window to new aspects of tumorigenesis

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ABSTRACT

Familial lentiginosis syndromes cover a wide phenotypic spectrum ranging from a benign inherited predisposition to develop cutaneous lentigines not associated with systemic disease to associations with several syndromes that carry an increased risk of formation of hamartomas, hyperplasias and other neoplasms. Within the last decade, the molecular pathways involved in the etiology of these syndromes have been more clearly defined and it now appears that several major cellular signaling pathways are involved; the protein kinase A (PKA) pathway in Carney Complex (CNC), the Ras/Erk MAP kinase pathway in LEOPARD/Noonan syndromes, and the mammalian target of rapamycin pathway (mTOR) in Peutz-Jeghers syndrome and the diseases caused by PTEN mutations. In this review, we discuss the clinical presentation of these disorders and provide an update on the molecular mechanisms involved. We speculate that the presence of lentigines in these diseases caused by diverse molecular defects is probably more than an associated clinical feature and likely reflects cross-talk and convergence of signaling pathways that are of central importance to embryogenesis, neural crest differentiation, and end-organ growth and function of a broad range of tissues, to include those of the endocrine, reproductive, gastrointestinal, cardiac system, and the integument systems.

Introduction

“…This combination of lesions is best explained by the concept of neurocristopathies” is what the medical examiner in rural Pennsylvania concluded finishing his report on the autopsy of a 19-year-old heavily freckled man who died in 1981 due to malignant, metastatic (to his brain) pigmented schwannoma. The young man had been in and out the National Institutes of Health (NIH) Clinical Center for a variety of ailments; he had first been diagnosed with a growth-hormone producing tumor but his investigation and treatment was complicated by the baffling concurrent diagnosis of testicular tumors and hypercortisolemia due to adrenal tumors [1]. It was clear that he was affected simultaneously by two rare endocrine conditions, acromegaly and Cushing syndrome and several physicians had noted his many “freckles” and other pigmented skin lesions, but his disease was not actually diagnosed until years later. In 1995, upon reviewing records of NIH patients, investigators came across his clinical description and it became obvious that he had Carney complex (CNC). CNC, belongs to a group of disorders that are now slowly but surely molecularly elucidated, the familial lentiginoses (Table 1).

The lentiginoses share as one of their most prominent clinical features multiple lentigines; the lentigen being a hamartomatous melanocytic lesion of the skin clinically almost identical to a freckle but histologically quite distinct [2]. Peutz-Jeghers syndrome (PJS) is the prototype of these diseases which are almost all inherited in an autosomal dominant manner, have a relatively high rate of de novo cases and predispose to a variety of neoplasms [2]. Laugier-Hunziker Syndrome (LHS), arterial dissections with lentiginosis (ADL), centrofacial and partial unilateral lentiginoses, LEOPARD and Noonan syndrome with lentigines (NSL) are other lentiginoses [2]. A number of related disorders may be associated with lentigenes: Ruvalcaba-Myhre-Smith or Bannayan-Zonnana syndrome (RMS/BZS), a condition allelic to Cowden disease (CD) [3], Schimke immunoosseous dysplasia [4], Mulvihill-Smith (MSS) [5], Watson Syndrome [6], and the McCune Albright syndrome (MAS) [7], the two types of neurofibromatosis and other phacomatoses [8], multiple endocrine neoplasia 2B (MEN 2B) and nevus phacomatosis pigmentovascularis (NPP) [9]. Xeroderma pigmentosum may also be associated with
solar lentigines [10]; a number of chromosomal conditions and syndromes predisposing to premature aging, immunodeficiency or associated with DNA and/or chromosomal repair defects may also present with lentigines. However, in most of these conditions, lentigines are either secondary or peripheral to the primary lesions and do not represent hamartomatous proliferation of the melanocytes as is the case in the familial lentiginoses. In this review, we focus on the latter, and among them, the syndromes that have been molecularly elucidated over the last decade: CNC, PJS/LHS, LEOPARD and NSL and the conditions caused by PTEN mutations (RMS/BZS and CD). The argument is made that the affected signaling pathways, protein kinase A (PKA), Ras/Erk MAP kinase, and the mammalian target of rapamycin (mTOR) converge to a complex system of cellular check and balances that oversee growth, proliferation, and differentiation of many cell types; their perturbation causes a wide array of manifestations, including neoplasms that range from the simple lentigen to aggressive malignancies.

**TABLE 1. The main lentiginoses: clinical manifestations and genetics**

<table>
<thead>
<tr>
<th>Disease</th>
<th>MIM</th>
<th>Clinical Manifestations</th>
<th>Inheritance</th>
<th>Locus</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carney Complex</td>
<td>160980</td>
<td>lentigines, PPNAD cardiac and skin myxoma schwannomas, acromegaly breast and testicular tumors</td>
<td>AD</td>
<td>17q22-24 (CNC1) PRKAR1A 2p16 CNC2 (?)</td>
<td></td>
</tr>
<tr>
<td>Peutz-Jeghers</td>
<td>175200</td>
<td>lentigines, GI polyps neoplasia (GI tract, pancreas breast, ovary, uterus)</td>
<td>AD</td>
<td>19p13.3 19q</td>
<td>LKB1/STK11 (?)</td>
</tr>
<tr>
<td>LEPARD</td>
<td>151100</td>
<td>lentigines, cardiac conduction abnormalities, aneurysms, pulmonic stenosis, cephalo-facial dysmorphism, short stature, sensorineural deafness, mental retardation, skeletal abnormalities</td>
<td>AD</td>
<td>12q22-qter</td>
<td>PTPN11</td>
</tr>
<tr>
<td>BRRS/CS</td>
<td>153480</td>
<td>macrocephaly, lipomatosis, pigmentation of the glans penis, mental retardation, vascular malformations</td>
<td>AD</td>
<td>10q23</td>
<td>PTEN</td>
</tr>
<tr>
<td>Lentiginosis</td>
<td>151001</td>
<td>lentigines (centrofacial, palmoplantar, trunk), as above in addition to mental retardation, skeletal dysraphia</td>
<td>AD</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td></td>
<td>151000</td>
<td></td>
<td>AD/sporadic</td>
<td>unknown</td>
<td>unknown</td>
</tr>
</tbody>
</table>
Lentigo

Lentigines are often divided into two broad categories, simple lentigo and solar lentigo. Macules of café-au-lait color that develop with older age are also called lentigo but they are clinically and genetically different lesions. In general, lentigines associated with the genetic diseases that are being discussed in this review develop at a young age, often increase in number during adolescence and are not restricted to sun-exposed areas, whereas solar lesions often develop after the third decade of life, increases with advancing age, and as the name implies, are found almost exclusively on sun-exposed areas [11, 12]. While the descriptive identification appears fairly straightforward, the clinical distinction of simple lentigo is at times more difficult, with lesions often confused with ephelides (freckles). However, several clinical features and histologic differences do exist and should aid the clinician in separating these lesions. Although both types are of similar size and appearance and are often described as multiple 4-10 mm, variegated, brown to black macules, lentigines typically do not darken with sun exposure (as compared to ephelides) and may be distributed on distinct anatomic locations such as the face (around the eyes and on the eyelids, the saddle of the nose, and the perioral areas of the upper and lower lips, crossing the vermilion border in some diseases), palmoplantar regions of the hands and feet, breast nipples, buttocks and genital region (labial, vaginal mucosa, prepuc and penile mucosa and skin), and less commonly other inner mucosal surfaces (buccal and anal mucosa, and/or the conjunctivae, especially the inner and outer canthal areas) [2, 3]. Histologically, while there is some overlap with freckles, lentigines show prominent rete ridges and melanocytic hyperplasia. In the case of ephelides and solar lentigines, increased pigmentation results from donation of pigment from otherwise normal (albeit stimulated) melanocytes to adjacent keratinocytes [2, 11, 12]. Clinically, lesions on mucosal surfaces -in particular the lacrimal caruncle of the eye- and lesions that cross the vermilion border of the lips are of particular importance as pigmented lesions found in these locations are often heralding features of the familial lentiginosis syndromes that are associated with systemic disease.

Benign lentiginoses

Centrofacial neurodysraphic [Mendelian inheritance in man (MIM) 151000] (13) and patterned (MIM 151001) lentiginosis describe two inheritable conditions that, in keeping with all of the familial lentiginosis syndromes follow autosomal dominant (AD) inheritance, but in contrast to the other members of this group are not associated with systemic disease [14, 15]. Touraine et al. described the first syndrome, centrofacial neuro-dysraphic lentiginosis, reporting on a group of 32 patients from 17 families with early onset of facial lentigines, occasionally found on the upper lip, that faded over time but was frequently associated with mental retardation [14]. In 1989, O’Neil et al., described inherited patterned lentiginosis: what distinguished this group of patients was the increased incidence in the African-American population and the description of lentigines not only limited to the facial region, but involving the hands, elbows and buttocks as well [16]. Interestingly, neither of these syndromes are associated with lesions of the oral mucosa [16, 17]. Several other reports of multiple lentiginoses syndromes have since been published, but the significance of these syndromes, and potential overlap with other previously described syndromes is as of yet unknown [17, 18].
Carney complex

The association of myxomas, spotty skin pigmentation (lentigines) and endocrine overactivity was first reported by Dr. J. Aidan Carney in 1985 and subsequently designated as CNC by Bain in 1986 (19) and Carney syndrome by MIM (13) in 1994. With the report of this new syndrome it was realized that the majority of patients previously characterized under the separate diagnoses of LAMB (lentigines, atrial myxoma, mucocutaneous myxoma, blue nevi) and NAME (nevi, atrial myxoma, myxoid neurofibroma, ephelide), would now be more appropriately described under CNC. The diagnosis of CNC is made if two of the main manifestations of the syndrome are present [20]; these need to be confirmed by histology, biochemical testing or imaging; alternatively the diagnosis is made when one of the criteria is present and the patient is a carrier of a known inactivating mutation of the PRKAR1A gene (see “molecular mechanism” for discussion) [20-22].

The most common features of CNC include spotty skin pigmentation (Figure 1) (lentigines, freckling, café-au-lait spots, and blue nevi), myxomas of the heart, skin, and breast, and primary pigmented nodular adrenocortical disease (PPNAD) associated with an atypical form of Cushing syndrome (CS) [23,24]. The breadth of involved organs in CNC is quite unique; CNC is both a multiple endocrine neoplasia (MEN) (along with MEN-1 and -2) and a cardiocutaneous syndrome (along with LEOPARD and similar conditions). Of the non-cutaneous lesions found in CNC, cardiac myxomas are the most common [22, 24]. These tumors tend to be of a more aggressive nature when compared to sporadic, non-CNC-associated myxomas: unlike the latter, the former may be in any cardiac chamber and may present multiple times, starting at a very young age (even in infancy) and without any predilection for gender (sporadic myxomas are more common in older women and almost always occur in the left atrium as single one-time tumors) [22]. Historically, cardiac myxomas have been reported to be responsible for more than 50% of the disease-specific mortality among CNC patients [24].

Endocrine gland involvement includes growth hormone (GH) secreting pituitary adenomas, thyroid gland disease, corticotropin (ACTH)-independent CS secondary to primary pigmented nodular adrenocortical disease (PPNAD), and testicular tumors, in particular, large cell calcifying Sertoli cell tumors (LCCSCT) [20-24]. Overall, PPNAD is the most common endocrine lesion and causes the greatest degree of endocrine-associated morbidity (discussed in more detail below) [24]. In male patients, however, the occurrence of LCCSCT may supersede PPNAD in number, but not in morbidity, as it is typically a benign lesion most often diagnosed during routine testicular ultrasound when microcalcifications are found [21, 24]. Leydig cell tumors and adrenal rests have also been reported [21]. Ovarian cysts are often found by sonographic examination as multiple hypoechoic lesions and although usually clinically insignificant they may progress, occasionally, to ovarian carcinoma [20, 26, 27].

Thyroid gland disease spans the spectrum from nodular disease to carcinoma, but in contrast to pituitary and adrenal pathology, there does not appear to be an increased risk of hyper- or hypothyroidism. By sonographic examination, more than 60% of children and adults with CNC will be found to have cystic or multinodular disease [28]. On biopsy, follicular adenoma is the most common finding, whereas thyroid cancer, follicular or papillary, may develop in up to 10% of CNC patients with preexisting thyroid pathology [21, 28]. Of note, recent examination for loss-of-heterozygosity (LOH) at the CNC locus on chromosome 17 (17q22-24) in sporadic thyroid cancer has found
increased loss of this region, supporting the hypothesis that thyroid tissue is susceptible to tumorigenesis induced by \textit{PRKAR1A} loss of function [29].

\textit{Molecular Mechanism}

Genetic linkage analysis has revealed two distinct loci for CNC, one on chromosome 2p16 (\textit{CNC2}) and the other on chromosome 17q22-24 (\textit{CNC1}) [30, 31]. Inactivating mutations of the gene encoding the protein kinase A type I-\(\alpha\) regulatory (R1\(\alpha\)) subunit (\textit{PRKAR1A}) were identified in patients mapping to the chromosome 17 and analysis of 53 of the 70 kindreds registered in the National Institutes of Health-Mayo Clinic collection has revealed that 28 out of the 53 (52.8\%) have mutations at the \textit{CNC1} locus [34]. The gene responsible for CNC at the chromosome 2p16 locus is unknown and there is at least one large kindred with CNC that does not map to either the \textit{CNC1} or \textit{CNC2} locus [34]. At this point, there are no clear phenotypic differences between families mapping to one or the other locus.

The role of R1\(\alpha\) in human tumorigenesis has been explored in several different cancer tissues and cell-lines. Enhanced expression of R1\(\alpha\) has been shown to play a role in colorectal, renal, breast, and ovarian cancer, and malignant osteoblasts, and may be associated with more advanced disease [32-39]. The notion of reduced R1\(\alpha\) activity had not been investigated prior to the discovery of it being the protein that was defective in CNC; CNC represents the first identified human disease associated with a mutation of the PKA heterotetramer. The majority of mutations in the \textit{PRKAR1A} gene result in premature stop codons, with the most frequent mutations found in exons 2, 4 and 6 [40]. Predicted mutant protein products are not found in affected cells secondary to nonsense mRNA mediated decay (NMD) of the mutant sequence [31]. Biochemically, loss of R1\(\alpha\) leads to increased cAMP-stimulated total (but not PKA-specific) kinase activity, [31, 41] thought to be secondary to up-regulation of other components of the PKA tetramer, including both type I (\textit{PRKAR1B}) and type II (\textit{PRKAR2A} or \textit{PRKAR2B}) subunits, in a tissue dependent manner [20] but how this leads to increased tumorigenesis is currently unknown.

Initial data supported the role of \textit{PRKAR1A} as a “classic” tumor-suppressor gene with tumors from CNC patients exhibiting germline mutations and subsequent LOH at the \textit{PRKAR1A} locus; however, it now appears that haploinsufficiency of \textit{PRKAR1A} may be sufficient for phenotypic expression of increased PKA activity [42] and the development of certain tumors, such as eyelid myxomas [43]. This concept is exemplified in animal models of CNC: whereas mice homozygous for R1\(\alpha\) deletions die early in utero [44], transgenic mice with heterogeneous expression of an antisense transgene for exon 2 of \textit{PRKAR1A} exhibit many of the phenotypic characteristics of CNC patients, including thyroid follicular hyperplasia and non-dexamethasone suppressible hypercortisolism [45, 46]. Not all of these lesions exhibited consistent losses of the normal R1\(\alpha\) allele [46].

Examination of the mechanisms associated with loss of R1\(\alpha\), increased PKA activity, and tumorigenesis are currently underway. PKA is a ubiquitous serine-threonine kinase intimately involved in the regulation of cell growth, including a potential role in chromosome stability [21]. The cross-talk between signal transduction pathways and the tissue specific effects of altered PKA function are inherently quite complex, reflected by at times conflicting data. For example, alterations of 17q and/or the \textit{PRKAR1A} locus have been found in both sporadic adrenal and thyroid cancers yet allelic loss of 17q in cardiac and skin myxomas from CNC patients, with known germline \textit{PRKAR1A}
mutations have not been found [21, 43]. Interestingly, CNC myxomas appear to have a more aggressive nature when compared to sporadic, non-CNC-associated myxomas, as discussed previously.

The physiologic impact of PRKAR1A-inactivating mutations has been most thoroughly studied in PPNAD, a rare form of ACTH-independent CS, which is present in approximately one third of CNC patients. PPNAD often presents in an indolent fashion and may be difficult to diagnose due to an intermittent or cyclical nature of the associated hypercortisolism [22]. Diagnosis is established using the six-day Liddle test as patients with PPNAD show a classic paradoxical rise in the 24 hour urinary free cortisol and/or 17-hydroxysteroids of more than 50% on the second day of high dose dexamethasone administration [24]. While this response appears to be pathognomonic for PPNAD it does not appear to be dependent on the presence of PRKAR1A mutations as comparative in vitro studies between PPNAD cell lines with and without R1α deficiency showed increased cortisol secretion in response to dexamethasone associated with increased expression of the glucocorticoid receptor [47]. The underlying mechanism for this response is not known, however.

Additional studies aimed at elucidating the inter-relationship between the PRKAR1A status, altered PKA activity and cellular metabolism are being aggressively pursued. Microarray analysis of R1α antisense targeted tumor cells has recently been shown to change expression of more than 240 genes suggesting that altered regulation of a significant number of downstream targets is likely to contribute to the CNC phenotype [48]. Investigation of one of the signaling pathways, the mitogen-activated protein kinase (MAPK) ERK 1/2 pathway, typically inhibited by PKA in many cells, has recently been reported. In this report, the lymphocytes from CNC patients with known PRKAR1A mutations showed altered PKA activity and increased ERK 1/2 phosphorylation [42]. Cell metabolism and cell proliferation studies suggested that altered PKA activity is associated with reversal of PKA-mediated inhibition of the MAPK pathway resulting in increased cell proliferation [42].

LEOPARD syndrome

LEOPARD is also often referred to as multiple lentigines syndrome (MLS); the acronym, that also describes the pattern of pigmentation (Figure 2), was suggested first by Gorlin et al in 1969 [50] and reflects the components of this cardiocutaneous disorder: lentigines, electrocardiographic conduction defects, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation of growth, and sensorineural deafness [51, 52]. The diagnosis is established if multiple lentigines are present in association with at least two other features; if lentigines are absent, a first-degree relative affected with LEOPARD syndrome and three of the other six features are needed for diagnosis [52].

As in CNC patients, the lentigines usually develop in childhood and are often the first clinical manifestation to appear [52]; they are located primarily on the face and upper trunk, less commonly involving the oral mucosa, extremities, genitalia or conjunctiva of the eye [53]. The appearance and distribution of the lentigines are very similar to CNC, as is the histologic appearance, including melanocytic hyperplasia and elongation of the rete ridges. One important difference is the absence of pigmented spots along the vermilion border of the lips, a finding that is characteristic in CNC and PJS patients. Also, skeletal abnormalities, onychodystrophy and hyperelastic skin that are often found in LEOPARD syndrome patients are not common in other lentiginoses [52, 53]. The craniofacial features of LEOPARD syndrome are generally coarse and include low-set and posteriorly rotated ears, hypertelorism with or without ptosis, webbed neck and mandibular prognathism [52]. These features,
combined with an increased incidence of pulmonic stenosis, show significant phenotypic overlap with Noonan syndrome (MIM 163950); furthermore, hyperelasticity and other features partially overlap with Ehlers-Danlos syndrome subtypes and the ADL syndrome (see below) [52]

Morbidity and mortality associated with LEOPARD syndrome are dependent on the extent of cardiac disease. Multiple congenital heart defects have been reported to include not only pulmonic stenosis (present in 40% of patients) but also subaortic and subpulmonic stenosis, and hypertrophic obstructive cardiomyopathy [53]. In addition, conduction abnormalities are common and whether they are a primary defect, or secondary to structural abnormalities, they may result in sudden cardiac death [54]. More recently a predisposition to widespread, recurrent polyaneurysms has been reported in a patient with genetically confirmed LEOPARD syndrome [55]. Although unproven, it is interesting to speculate whether patients previously diagnosed with the ADL disorder (OMIM 600459) may be more appropriately reclassified as a variant of LEOPARD syndrome; to our knowledge, these patients have not had genetic testing [56].

Molecular mechanism

The clinical similarities between LEOPARD and Noonan syndrome (NS), and the series of patients with both lentigines and NS-like features (NSL) raised the question whether these syndromes could be allelic disorders with varied clinical expression. Some of the patients with NSL had in fact Watson syndrome, a condition that is allelic to neurofibromatosis type-I. Still, LEOPARD, NS and NSL share similar craniofacial features, an increased incidence of sensorineural deafness and cryptorchidism, and associated pulmonic stenosis. In total, up to 10% of NS patients have lentigines [57]. In 1996, mutations in the \textit{PTPN11} gene (12q24.1), encoding the non-receptor tyrosine protein tyrosine phosphatase Shp-2 (Src homology 2 domain-containing protein tyrosine phosphatase-2) were found to be the cause of NS in 50% of the patients [58]. In 2002, independent research groups published reports linking \textit{PTPN11} mutations to LEOPARD syndrome [59, 60].

Shp-2 is an important intermediate in several signaling pathways involved in modulating cellular proliferation, differentiation and migration. Vertebrates have two Shp proteins, Shp-1 and Shp-2, both having two N-terminal domains, N-SH2 and C-SH2, a catalytic protein-tyrosine phosphatase (PTP) domain and a C-terminal tail [61]. Shp2 is a key-regulatory protein in the receptor tyrosine kinase (RTK) signaling pathways whose primary role is to down-regulate GTPase activating proteins (GAP) resulting in activation of Ras [61]. In the basal state, the N-SH2 domain of Shp-2 exhibits an inhibitory role over the phosphatase activity by allosterically blocking the binding domain of PTP [61]. To date, the \textit{PTPN11} mutations that have been described in both NS and LEOPARD are believed to be gain of function mutations leading to dysregulated phosphatase activity with subsequent increased inhibition of GTPase which in turn leads to increased Ras activity [61, 62]. In keeping with this, \textit{in vitro} studies of hematopoietic cell lines harboring \textit{PTPN11} mutations show increased proliferation rates and reduced growth factor requirements [62].

How these abnormalities result in human disease and what intermediary molecules and additional down-stream targets of Shps are affected by mutations of the \textit{PTPN11} gene are as of yet poorly understood. It is of interest to note, however, that SHP-2 has mitogenic effects on vascular smooth muscle and interacts with several key elements of angiogenesis, including the angiopoietin-1 receptor and the signaling cascade of vascular endothelial growth factor (VEGF) [54].
Accumulating evidence suggests that certain germline PTPN11 mutations play a key role in certain manifestations of LEOPARD, NS and/or NSL, such as the malformations of the cardiovascular system and predisposition to certain malignancies [54]. For example, exon 8 mutations were more commonly associated with pulmonary valve stenosis, exon 7 and 12 mutations were more frequently associated with hypertrophic cardiomyopathy, whereas those of exon 3 were associated with atrial septal defects [63]. Certain mutations are also associated with malignancies in both the germline and somatic state: although neoplasms are uncommon in LEOPARD patients, children with NS have in increased incidence of hematologic disorders, including juvenile myelomonocytic leukemia (JMML) [62]. Recent studies indicated that in addition to mutations in the Ras oncogene and inactivating mutations of NF1, in a mutually exclusive manner, 25 to 30% of JMML cases harbored somatic PTPN11 mutations [62, 64]. These mutations are found in similar locations as those found in LEOPARD and NS, but resulted in different amino acid substitutions [61]. This apparent genotype-phenotype correlation was further supported by the observations made in a transgenic mouse model which expressed a heterozygous NS-associated PTPN11 mutation (D61G) developing short stature, craniofacial abnormalities, myeloproliferative disease and multiple cardiac defects [65].

**Peutz-Jeghers syndrome**

PJS is a disorder characterized by mucocutaneous lentigines (lips, buccal mucosa), gastrointestinal (GI) hamartomatous polyps (affecting the small bowel, stomach and large colon) and an increased risk of developing early onset adenocarcinoma of the GI tract, as well as tumors of the pancreas, breast, thyroid and reproductive organs [66, 67]. There is significant clinical overlap between PJS and CNC to the point that some patients with CNC in the NIH series had been diagnosed with PJS (the opposite is less frequent since PJS is a widely known condition, whereas CNC was only recently described). As in CNC, patients with PJS may have lentigines of the lips, buccal mucosa, genitals, or the hands and feet (Figure 3), which tend to fade in the older age [67] (an important consideration in the evaluation of the older patient with multiple hamartomatous GI polyps) and a number of other skin lesions (mostly compound but also blue and Spitz nevi), thyroid tumors and an increased incidence of gonadal tumors. Most males with PJS, as patients with CNC, have, upon sonographic examination, testicular microcalcifications which reflect the presence of multiple foci of LCCSCTs. Leydig cell tumors are less frequent, as in CNC. LCCSCTs may express aromatase and lead to precocious puberty and gynecomastia [66-68], clinical findings that are more common in boys with PJS than in those with CNC. Females with PJS develop a variety of gynecological neoplasms; their high prevalence and histologic subtypes are at variance with those of women with CNC [27].

It was recently reported that the cumulative life-long risk for developing cancer in a patient with PJS exceeds 90% [66]. There does not appear to be a risk difference between genders, with the exception of gonadal malignancies and breast cancer, which are far more common in females [69]. The mean age of diagnosis of a first cancer was 42.9 ± 10.2 years in one study [63]. The most recent study of 240 patients with PJS (188 familial and 52 sporadic cases were included), all with confirmed genetic defects, showed an age-dependent risk of developing cancer: 1% at age 20, 3% at 30, 19% at 40, 32% at 50, 63% at 60 and 81% at 70 years [69]. Overall, 54 malignancies were diagnosed in 47 carriers; GI (esophageal, stomach, small bowel, colon, rectum and pancreas) and breast cancers were the most common. Compared to the general population, the risk of developing colorectal and breast cancer in PJS by age 60 was 42% and 32% vs. 1% and 5%, respectively [69]. Thus, breast cancer risks in
women with PJS are comparable to those of women with either \textit{BRCA1} or \textit{BRCA2} mutations. The optimal time to initiate GI and breast cancer surveillance is still somewhat controversial, although most agree that GI screening (by endoscopy, colonoscopy and abdominal CT) and breast exams with reproductive tract screening (by pelvic ultrasound, cervical cytology and serum CA125 levels) should start after age 20 and 25, respectively [69].

\textit{Molecular mechanism}

In 1998, two independent reports identified germline mutations in the gene \textit{LKB1/STK11} on 19p13.3, coding for a serine/threonine kinase, as the cause of PJS in most (but not all) patients [70, 71]. To date mutations in \textit{LKB1} can be found in only 30-80\% of patients; linkage to other loci, including 19q13.4 has also been reported but the causative gene(s) have not bee identified [72, 73]. \textit{LKB1} appears to function as a classic tumor suppressor following Knudson’s two-hit hypothesis, at least as far as the development of GI neoplasms is concerned. Interestingly, \textit{LKB1} also appears to play a role in two additional key-regulatory pathways, involved in controlling the polarity of epithelial cells and as the master regulator of AMP-dependent kinase, the central sensor of cellular ATP levels and key regulator of cellular energy consumption [74-77]; \textit{LKB1} may also be interacting with PRKAR1A, and is phosphorylated by PKA (Figure 4A).

\textit{The lentiginoses and the mTOR Pathway}

It has recently been suggested that \textit{LKB1} defects lead to dysregulation of the downstream target mTOR, the mammalian target of Rapamycin, a key regulator of protein translation [78, 79]. mTOR is a highly conserved serine/threonine kinase that mediates cellular growth by sensing information on the cellular energy status and mitogenic signals and then coordinating the activity of the translational machinery of the cell through regulation of the ribosomal protein S6 kinases (S6Ks) and the eukaryotic translational initiation factor 4E (eIF4E)-binding proteins (4E-BPs) [80]. Dysregulated activation of mTOR is believed to allow growth of cells to occur at times of reduced nutrient or energy supply and independently of mitogenic stimuli with the “uncoupling” hypothesized to be the underlying mechanism responsible for the formation of hamartomas and neoplasia in PJS and other conditions (Figure 4B). LKB1 plays a fundamental role in regulating cellular energy metabolism by down-regulating mTOR-dependent protein synthesis during times of nutrient stress [81]. This regulatory control is mediated through the direct activation of AMP-activated protein kinase (AMPK), a primary sensor of cellular response to reduced ATP levels. AMPK is activated by a variety of stimuli, including oxidative and osmotic damage, hypoxia and hypoglycemia [81]. Once activated, AMPK phosphorylates and activates tuberin (encoded by the tumor suppressor \textit{TSC2} and mutated in tuberous sclerosis type-II), resulting in inhibition of mTOR signaling [82]. At the basal state, LKB1 protects cells from apoptosis by reducing protein synthesis at times of stress. Loss of \textit{LKB1} function results in dysregulated mTOR mediated protein synthesis [74-79]; in addition, aberrant \textit{TSC1/TSC2} and/or mTOR signaling in these cells results in increased angiogenesis through activation of hypoxia inducible factor 1 (HIF) and VEGF [83]. Consistent with the above, \textit{Lkb1}-deficient mice develop intestinal hamartomatous polyps [84, 85], hepato-cellular carcinoma [86]. Interestingly, \textit{Lkb1}-deficient cells are resistant to \textit{Ras}-induced transformation [84], as would have been expected from a disruptor of mTOR signaling. While these alterations shed light on aberrant cellular metabolism and may explain why cells without normal \textit{LKB1} function show immortalized growth and decreased apoptosis they do not fully explain the apparent tumorigenic affect of \textit{LKB1} mutations. Further study of the \textit{LKB1}
pathway and its interaction with other signaling molecules, including the LKB1-specific adaptor protein STRAD, and other intermediaries (PAR1, PKA, or any of the 13 or more additional kinases of the AMPK subfamily) will need to be completed to more fully understand how LKB1 choreographs cellular organization and growth [74, 76, 87].

**Laugier-Hunziker syndrome**

Laugier-Hunziker syndrome (LHS) is a rare, sporadic disorder, originally described in 1970, that is often confused for PJS due to similar appearance and distribution of hyperpigmented cutaneous and mucocutaneous lesions [88, 89]. Family history and screening for mutations may not aid in distinguishing this disorder from PJS as up to 25% of PJS cases are sporadic, and as previously reviewed, LKB1 mutations may be found in only 30-80% of cases [75]. Accurate clinical diagnosis is essential as patients with LHS are not at an increased risk of developing GI tumors and they do not need any invasive GI tract surveillance [89].

**Ruvalcaba-Myhre-Smith, Bannayan-Zonnana syndrome, and Cowden disease (CD)**

RMS/BZS, CD along with PJS and juvenile polyposis are a group of inherited disorders that have been previously grouped under the general classification of the familial hamartoma syndromes. With the discovery of mutations in the tumor suppressor gene PTEN (10q22-q23) in up to 80% of CD patients and up to 60% of RMS/BZS patients, it has been suggested that these conditions should be all listed under the heading “PTEN hamartoma tumor syndromes” (PHTS) [90-100]. Patients with PTEN mutations have an increased risk of developing multiple hamartomas in various organ systems such as the breast, thyroid, skin, central nervous system, and the GI tract [98]. Some distinguishing features of RMS/BZS include delayed motor development, and most germane to our discussion, the presence of lentigines, especially on the glans penis known as the “speckled penis” [98]. The association of macrocephaly, lipomatosis and speckled penis is also known as the Bannayan-Riley-Ruvalcaba triad [96]. Hyperpigmentation of the glans penis typically develops during childhood; in 90% of CD patients, mucocutaneous signs develop by 20 years of age [98-100]. Diagnostic criteria for PHTS, and a thorough review of this topic, were recently published [101]. It should be noted that PTEN mutations have also been found in several other conditions, including Proteus syndrome (PS) (MIM 176920) and Proteus-like disorders [102-104], Lhermitte-Duclos disease (LDD), and other rare syndromes [105-106].

**Molecular Mechanism**

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a dual-specific phosphatase that plays a key role in cell growth, differentiation, apoptosis, membrane trafficking, cellular interactions and cellular motility.[99, 107, 108] The mechanics of how PTEN regulates such diverse and key regulatory pathways has been the focus of intense research as early on it was recognized that inactivation of PTEN/MMAC (mutated in multiple advanced cancers) effected a large number of cancers and appeared to correlate with advanced disease in CNS glial tumors (glioblastoma multiforme and anaplastic astrocytoma), advanced prostate cancer and in metastatic breast cancer [107, 108]. The early reports of PTENs inhibitory regulation of the phosphoprotein focal adhesion kinase (FAK) and its effects on decreasing cell spreading and motility have since given way to the ever increasing body of research on the regulator role of PTEN on the phospholipids, and of particular
interest, the ability of PTEN to regulate the plasma membrane inositol phospholipids functioning as the “off” switch for the phosphoinositide 3-kinase (PI3K) signaling pathway [107-109]. It has now been suggested that loss of PTEN activity leads to constitutive activation of the cytosolic signaling protein AKT [109]. One of the key downstream targets of AKT is the tuberin-hamartin complex (TSC1/TSC2), mutations of which are associated with the hamartomatous syndrome tuberous sclerosis [110]. Early evidence suggests that the tuberin-hamartin complex, through inhibition of mTOR signaling, inhibits the 70 kDa ribosomal S6 kinase (S6K1) and eukaryotic initiation factor 4E-binding protein (4E-BP1), key mediators of the protein translational machinery [110]. In this model, loss of PTEN function results in the constitutive activation of AKT, downregulation of tuberin/TSC2 and mTOR and subsequent promotion of cell-cycle progression and suppression of apoptosis. Elucidation of this pathway, mediated through mTOR, now provides a link for the previous observation linking the role of mitogenic stimuli in breast, colon and prostate cancer as well as in the hamartomatous tumor syndromes PJS, TS and CD [107]. A more thorough review of the role of mTOR and translation in cancer pathogenesis has recently been published [111-114]. The role of the bone morphogenetic proteins (BMP) in regulating PTEN levels was also recently revealed [115-117]. Bmpr1a-mutant mice develop intestinal polyps, an effect that is largely due to loss of BMP inhibition of PTEN function and to some extent Wnt-signaling cross-talk [117, 118].

Summary: lentigines, a clinical sign of molecular convergence?

Although a lot remains to be learned, there is compelling evidence to suggest that the apparently different pathways that result in the main lentiginoses are also involved in melanocytic differentiation and migration. Under the direction of several key regulatory signals, including those from the Wnt family of proteins, fibroblast growth factors and BMPs, neural crest cells differentiate along the dorso-lateral pathway that gives rise to the melanocytes [119-121]. Interactions with factors such as the microphthalmia-associated transcription factor (Mitf), mTOR, and possibly BRAF and the dickkopf proteins (DKK) may provide molecular basis for why the melanocyte is affected in such a way in these disparate disorders. Figure 4A and B is a summary of what we learnt in the last 10 years about the lentiginoses, but still a far way from knowing everything we should know!

REFERENCES


13.


FIGURE LEGENDS

Figure 1. Pigmentation in Carney complex. A. A blue nevus on the cheek of a patient with the complex; B. Lentigines on the sides of the nose; C. Lentigines on the vermillion border of the lips and the mucosa; D. Blue nevus on the dorsal surface of the hand. Unusual pigmented lesions in patients with Carney complex may occur everywhere and are not unusual even in newborns with the disease.

Figure 2. Features of Noonan/LEOPARD syndromes. A. Pigmented lesions on the face can mimic those of patients with Carney complex, but they tend to not be present on the lips or periorally; they occur mostly as single darkly pigmented spots and look less like freckles; B. Lentigines on the shoulders and the back can be extensive; winged scapulae is also an evident deformity in this patient; C. Skeletal deformities, scoliosis and hyperextensibility, like that shown in this image, are not unusual in patients with this condition.

Figure 3. Pigmentation in Peutz-Jeghers syndrome looks similar to that in Carney complex except that single lesions tend to be larger and more pigmented. A. Lentigines on the vermillion border of the lips and a darkly pigmented lesion on the mucosa (arrow); B. Freckling around the eyes with multiple lentigines; C. and D. Pigmented lesions in the oral mucosa are frequent in patients with this syndrome, perhaps more frequent than in any other lentiginosis.

Figure 4A: Two independent pathways appear to be critically important in regulating cell growth in response to nutrient supply and mitogenic stimulation: 1) the PKA/PRKAR1A - LKB1 tumor-suppressor protein pathway, acting via AMPK, and 2) the PI3K/AKT pathway. Recent evidence suggests that the tumor suppressor gene complex, TSC1/TSC2, orchestrates the signal from both pathways to the downstream target, mTOR, which in turn regulates the ribosomal protein S6 and 4EBP-1, a repressor of the translational initiation factor eIF4E. In this model, at times of nutrient stress LKB1/AMPK activation of the TSC1/TSC2 complex results in inhibition of mTOR and a decrease in protein synthesis. Under stimulation of mitogenic pathways, PI3K phosphorylates PIP2 to PIP3 resulting in recruitment of AKT to the membrane where it is activated by PDK1. Activated AKT inhibits the TSC1/TSC2 tumor suppressor complex leading to increased mTOR activity. In the later pathway, PTEN antagonizes PIP3 action through dephosphorylation, and thus provides an ‘off’ switch for regulating mitogenic pathway-induced cell cellular growth and proliferation. Cross-talk of several other pathways appears to play important regulatory roles in the lentiginoses syndromes to include the Ras/MAPK pathway in the regulation of translation, the LKB1 pathway in cellular polarity, the AKT pathway (as well as the TSC1/TSC2 complex) in the regulation of the Wnt/GSK3β/β-Cat pathway and the BMP pathway in the regulation of PTEN (see text for further discussion). Lastly, both PTEN and mTOR appear to have negative regulatory effects on VEGF through loss of stabilization of the hypoxia inducible transcription factor 1 (HIF1).

Figure 4B: Loss of function of the tumor-suppressor proteins LKB1 (or its regulator PKA/PRKAR1A) in Peutz-Jeghers syndrome, PTEN and BMP in Cowden syndrome/Banayan-Riley-Ruvalcaba (or TSC1/TSC2 in tuberous sclerosis) leads to a proliferative advantage for the affected cells by switching on the protein translational machinery of the cells which advances cellular growth. In addition, LKB1 is required to maintain an organized, polarized epithelium in a cell-to-cell
interaction-independent manner; loss of LKB1 leads to loss of cellular polarity and loss of PTEN function to increased levels of PIP3, increased AKT, mTOR, β-catenin, and HIF activity. Accumulating evidence suggests that dysregulated activity of mTOR and the downstream effectors S6K1, 4EBP1, and HIF, as well as down-regulation of the GSK3b complex with increased β-Cat activity may be the triggering mechanism for hamartoma formation in the aforementioned syndromes.
The lentiginoses: Cutaneous markers of systemic disease and a window to new aspects of tumorigenesis
Andrew J Bauer and C A Stratakis

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