Inherited disposition to cardiac myxoma development

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Abstract | Carney complex is a genetic condition in which affected individuals develop benign tumours in various tissues, including the heart. Most individuals with Carney complex have a mutation in the *PRKAR1A* gene, which encodes the regulatory R1α subunit of protein kinase A — a significant component of the cyclic-AMP signalling pathway. Genetically engineered mutant *Prkar1a* mouse models show an increased propensity to develop tumours, and have established a role for R1α in initiating tumour formation and, potentially, in maintaining cell proliferation. Ongoing investigations are exploring the intersection of R1α-dependent cell signalling with other gene products such as perinatal myosin, mutation of which can also cause cardiac myxomas.

Carney complex (CNC) is an autosomal-dominant syndrome in which cardiac and extracardiac myxomas occur in the setting of spotty skin pigmentation and endocrinopathy. Although syndromic cardiac myxomas were initially described nearly half a century ago[1–5], more recent descriptions by Carney and others[6,7] have led to the disorder's eponymic nomenclature. Molecular genetic analyses[8] have demonstrated that mutations in *PRKAR1A*, which encodes the regulatory subunit type 1A (R1α) of the cyclic AMP (cAMP)-dependent protein kinase A (PKA), are responsible for disease in two-thirds of patients with CNC. Although *PRKAR1A* has been thought to function as a canonical tumour-suppressor gene in CNC, new findings indicate the potential for more complex mechanisms of CNC tumorigenesis[8]. The recent identification of a missense mutation in *PRKAR1A* that results in an enzymatically active mutant protein[8] sheds doubt on whether haploinsufficiency is the sole mechanism for tumour formation in CNC. Furthermore, other analyses demonstrate that mutations in the non-PKA phosphorylated perinatal myosin isoform (encoded by *MYH8*)[9] also lead to a CNC-like cardiac myxoma syndrome. Key questions that have so far evaded answer include the role of loss of heterozygosity (LOH) of *PRKAR1A* and *MYH8*, the role of PKA isozyme switching in tumour formation, relevant downstream targets of *PRKAR1A* in tumorigenesis and the complete identification of other genes that when mutated can also cause CNC.

Clinical manifestations of CNC

Cardiac myxomas are the most common primary heart tumours in adults; they account for nearly half of all primary cardiac neoplasms[10,11]. They are benign, slowly proliferating lesions of sub-endocardial origin, and the putative 'reserve' or 'lepidic' cells that give rise to the tumour are considered to be multipotent mesenchymal cells[7,12–15]. Histologically, tumours consist of polygonate cells (arranged singly or clustered) that are scattered throughout a proteoglycan matrix. Macroscopically, they appear as polypoid tumours, with a smooth or gently lobulated surface. Clinical symptoms depend on their size, location and mobility, and can include cardiac valve obstruction (shortness of breath (dyspnea) on exertion, palpitations, malaise and loss of consciousness (syncope), embolism (pulmonary embolism, stroke and myocardial infarction) and systemic rheumatic complaints (such as fatigue, fever, joint pain (arthralgia) and muscle pain (myalgia)) owing to the secretion of interleukin (IL)-6 by the tumour[12].

At least 7% of all cardiac myxomas occur in the setting of CNC[12] and are a cardinal feature of this disease. Based on histological examinations, there are no differences between sporadic and familial cardiac myxomas, although there are several differences in presentation and prognosis (TABLE 1). Sporadic cardiac myxomas usually occur in women of middle age (approximately 40–60 years old) and are generally isolated, single lesions of the left atrial aspect of the interatrial septum that do not recur after surgical resection. Familial cardiac myxomas show no age or gender preference, can occur in any intracardiac location, and can be single or multiple. Moreover, patients with familial cardiac myxomas often develop recurrent tumours despite adequate surgical resection of the initial lesions[7,16]. Such recurrent tumours are thought to reflect

Autosomal-dominant
Autosomal-dominant inheritance refers to genetic conditions that occur when a mutation is present in one copy of a given gene (in other words, the person is heterozygous).

Myxomas
A benign neoplasm of small stellate cells against an extensive proteoglycan background.

Endocrinopathy
A disorder that affects the function of an endocrine gland.

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At a glance

- Cardiac myxomas and various non-cardiac neoplasms occur in people who have the autosomal-dominant disorder Carney complex, which is also characterized by spotty pigmentation of the skin and endocrinopathy.
- Although complete penetrance is the rule, Carney complex shows a highly variable phenotype.
- Mutations in the chromosome 17q *PRKAR1A* gene, which encodes the regulatory R1α subunit of protein kinase A (PKA), cause Carney complex in approximately two-thirds of affected individuals. No genotype–phenotype correlation has been established, and most mutations result in *PRKAR1A* haploinsufficiency through nonsense-mediated degradation of the transcribed, mutant mRNAs.
- The contributions of loss of heterozygosity of *PRKAR1A* and increased PKA activity to Carney complex are unclear. Although both can occur in Carney complex tumours, analyses of human and murine tissues demonstrate that neither is required for tumorigenesis.
- Changes in the ratio of type I to type II PKA isoenzymes are uniform features of human Carney complex tumours, as well as tumours that are found in genetically engineered mouse models of Carney complex. Such PKA isoform switching might mediate altered cell growth and tumorigenesis.
- Mutation of the chromosome 17p *MYH8* gene, which encodes perinatal myosin, results in a rare familial cardiac myxoma syndrome with features that are typical of Carney complex, except that affected individuals also suffer from the hereditary distal arthrogryposis syndrome, trismus–pseudocamptodactyly.
- The mechanism by which *PRKAR1A* and *MYH8* mutations foster the survival and proliferation of myxoma progenitor cells in the heart remains unknown.

independent tumorigenic events rather than regrowth of or metastasis from the previous lesion, given their occurrence at remote sites at long periods after complete resection of well-encapsulated primary tumours.

In addition to cardiac myxomas, patients with CNC can develop myxomas in other sites. The most commonly diagnosed are cutaneous myxomas[6,8,17,18]. These skin tumours are usually benign and asymptomatic, and are localized in the upper dermis, dermis, and sub-cutis or the subcutaneous layer. They have a widespread distribution and can occur at any site, but have a particular predilection for eyelids, ears and nipples[18]. Another benign dermatological neoplasm that is seen in CNC is trichofolliculoma[19]. However, the most common skin findings in CNC are not neoplasms but benign lentigines and ephelides (freckles). Spotty pigmentation of the skin — which particularly affects unusual sites such as the urogenital mucosa, gingival borders of the lips, and the eye (sclera/cornea) — is the most consistently identified clinical feature of CNC and is present in more than 95% of patients with CNC[20]. Other CNC dermatological findings include blue naevi and café-au-lait spots.

Trichofolliculoma
A usually solitary tumour in which multiple abortive hair follicles open into a central cyst or space opening on the skin surface.

Lentigines
Benign brown pigmented macule with microscopic rete ridge proliferation.

Cushing syndrome
A disorder that results from increased adrenocortical secretion of cortisol.

Acromegaly
Endocrine disorder that is marked by progressive enlargement of peripheral parts of the body, especially the head, face, hands and feet, owing to excessive secretion of somatotropin.

Table 1 | **Clinical features of sporadic versus familial cardiac tumours**

Characteristics	Sporadic	Familial
Age	Middle age (approximately 40–60 years old)	Any age
Gender	Female	Male and female
Location	Left atrium	Predominantly left atrium, but any cardiac chamber can be affected
Recurrence	No	Yes

Endocrinopathy is an important feature of CNC. Individuals with CNC-related endocrinopathy often present with an unusual form of Cushing syndrome, primary pigmented nodular adrenocortical disease (PPNAD)[21]. True endocrine neoplasms in CNC include pituitary adenomas and thyroid tumours. Ten percent of patients with CNC develop growth hormone or prolactin-secreting adenomas of the pituitary gland. Because of the excessive production of growth hormone, these patients can present with acromegaly and gigantism[22], but these tumours are more often asymptomatic, sub-clinical lesions. Some patients with CNC will develop thyroid tumours such as follicular adenoma and/or papillary and follicular carcinomas[22].

Various other tumours have also been reported in some cases of CNC[23]. Large-cell-calcifying Sertoli cell tumour is a rare form of testicular tumour that occurs in about 50% of the male patients with CNC. They are sex cord stromal tumours that frequently present as bilateral and multifocal lesions with a low malignant potential[24,25]. Individuals with CNC might also show an unusual benign neoplasm, psammomatous melanotic schwannoma[26,27]. Other tumours have been suggested to occur at increased frequency in CNC, including breast fibroadenoma, ovarian cysts and tumours, gastric carcinoma, colon polyps and osteochondroma[28–31].

PRKAR1A mutation and CNC

Initial genetic evaluation of the families of individuals who are affected by CNC showed that the disorder was transmitted in a mendelian fashion as an autosomal-dominant trait with variable expressivity but almost complete penetrance. So, CNC kindreds were particularly amenable for linkage analyses. Initial reports indicated a potential locus on chromosome 2p (REF. 32), but subsequent reports demonstrated rigorous statistical evidence that a locus on 17q accounted for CNC in most CNC families[33], including ones that were previously associated with chromosome 2p. Subsequent positional cloning studies demonstrated that patients with CNC had mutations in the *PRKAR1A* gene at the chromosome 17q locus[34,35] (FIG. 1). *PRKAR1A* was initially described as the *TSE* (tissue-specific extinguisher) locus for its ability to suppress gene transcription in hepatic-derived cells[36,37]. These early analyses of the *TSE* locus showed that this gene suppression was mediated by phosphorylation of cAMP-response-element-binding protein (CREB), and the role of altered CREB-dependent gene transcription in CNC pathogenesis remains an important area of investigation. R1α exists as a dimer of regulatory subunits that interacts with the catalytic subunits of PKA and maintains the enzyme in an inactive form as a holotetramer[38] (FIG. 2). R1α is comprised of a dimerization domain, a hinge domain and two cAMP-binding domains (FIG. 1b). On cAMP-binding and saturation of both the R1α subunits, the PKA macrocomplex dissociates and activates the PKA catalytic subunits[39–41] (FIG. 2b).

In a recent analysis of 51 patients with CNC, 65% of patients screened were found to have a mutation in *PRKAR1A*[8]. In this study and others[42], no genotype–phenotype correlations were seen, and with a single

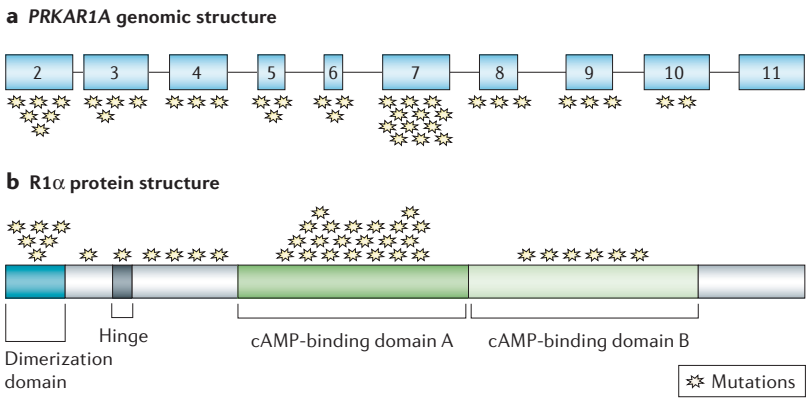

Figure 1 | ***PRKAR1A* mutations in CNC a** | Distribution of Carney complex (CNC)-associated mutations in the *PRKAR1A* gene, which encodes the regulatory subunit type 1A (R1α) of the cAMP-dependent protein kinase A. Indicated along the schematic of the *PRKAR1A* genomic structure are the locations of mutations that have been identified in patients with CNC. The dispersal of CNC mutations on an exon by exon basis highlights the random and even spread of mutations in the *PRKAR1A* gene. **b** | Distribution of CNC mutations in the R1α protein. Indicated along the schematic of the R1α protein structure are the locations of PRKAR1A mutations that have been identified in patients with CNC. Unlike the random distribution of CNC mutations when mapped on the *PRKAR1A* gene, there is an uneven clustering of mutations within cAMP-binding domain A. This bias of mutations to the cAMP-binding domain A might hint at the functional importance of this site.

exception (a missense mutation), all *PRKAR1A* mutations described to date are nonsense or frameshift (insertions, deletions or splice-site modifications) mutations. These are postulated, as described below, to lead to *PRKAR1A* haploinsufficiency through nonsense-mediated decay (NMD) of mutant mRNAs[8,42]. Although two mutation hot-spots, del(TG)576-577 and C769T have been described[8], *PRKAR1A* mutations are randomly distributed across all exons (FIG. 1a). The lack of genotype–phenotype correlations and the random mutation distribution are consistent with there being a single, common pathogenic mechanism underlying most mutations — haploinsufficiency[8].

However, it is notable that these generalizations about mutation distribution based on the genomic structure of *PRKAR1A* might be misleading. The distribution of mutations can also be considered relative to the four functional/structural domains of the R1α protein. Considered in this light, there is a statistically significant preponderance of mutations in the cAMP-binding domain A[8] (FIG. 1b). Assuming that such a predilection is not a random event and that the large genomic segment that comprises cAMP-binding domain A is not unusually susceptible to mutation, the high frequency of mutations in this domain is surprising if *PRKAR1A* haploinsufficiency is the sole mechanism of disease causation. Such a clustering of mutations suggests that cAMP-binding domain A mutations might have a specific phenotype that is clinically evident. Such clinical significance would be consistent with biochemical studies that demonstrate that, unlike cAMP-binding domain B, cAMP-binding domain A is required for high-affinity binding to PKA catalytic subunits and is indispensable[41,43,44]. Of course, such a domain-specific hypothesis of pathogenesis would also imply two points that have not been observed: a genotype–phenotype correlation, and a differential mechanism of action amongst putative haploinsufficient mutations. Future studies will need to be vigilant for both of these scenarios because they might be beyond the resolution of our current phenotyping and biochemical studies.

It is currently thought that most CNC mutations[8,34,35,45] lead to haploinsufficiency through an identical pathway of NMD. The NMD system is a well described cellular pathway[46,47] that permits cells to recognize aberrant transcripts and degrade them. In the setting of a heterozygous mutation that is recognized by NMD-sensing systems within mutant transcripts, the end result is degradation of most mRNA that is transcribed from the mutant allele and an effective halving of the gene dosage. The NMD system is efficient at recognizing premature stop codons that have been introduced by either nonsense or frameshift mutations in any coding exon, with the exception of the final and penultimate ones. So, in the case of *PRKAR1A*, most mutant transcripts are recognized through NMD and degraded, which results in true haploinsufficiency. Consequently, the levels of R1α protein are half the levels of those in normal cells[8,34,35,45–49].

To date, only five *PRKAR1A* mutations have been identified that evade NMD[8,45]. Three of these are mutations that occur in the final or penultimate exon and, therefore, exceed the boundaries of NMD surveillance[8]. However, these three mutations still result in haploinsufficiency as they seem not to encode a mature protein, possibly because of misfolding of the encoded protein in the endoplasmic reticulum and consequent degradation. One mutation[45], a G–T transversion in the 5′ splice-donor site of intron six, produced a truncated mRNA, and investigation of peripheral lymphocytes indicates that R1α protein might be produced from this transcript[45]. A single missense mutation (R74C)[8], the mutant mRNA of which escapes NMD and is expressed as mature protein, has also been identified. To date however, individuals with the R74C *PRKAR1A* mutation seem to have a CNC phenotype that is indistinguishable from that produced by *PRKARIA* haploinsufficiency, and the mechanism of action of the R74C mutation remains unknown.

Alternative mechanisms for CNC tumorigenesis

In the studies described above, approximately one-third of patients with CNC do not have detectable *PRKAR1A* mutations[8]. Previous linkage studies have demonstrated that the disorder is a genetically heterogeneous disease[32,50,51]. To identify other genes that cause CNC besides *PRKAR1A*, investigators have focused genetic analyses on CNC families that are not linked to chromosome 17q24.

We recently evaluated a large family in which typical CNC disease characteristics co-segregated with another mendelian phenotype, the autosomal-dominant distal arthrogryposis disorder trismus–pseudocamptodactyly syndrome (TPS), also referred to as Hecht–Beal syndrome. Individuals in this family had no detectable *PRKAR1A* mutation, and the family was not linked to either chromosome 17q24.1 or to chromosome 2p.

Trismus–pseudocamptodactyly syndrome
Rare inherited disorder that is characterized by the inability to completely open the mouth (trismus) and/or the presence of abnormally short muscle-tendon units in the hands and feet, causing the digits to curve or bend when the hand or foot is dorsiflexed.

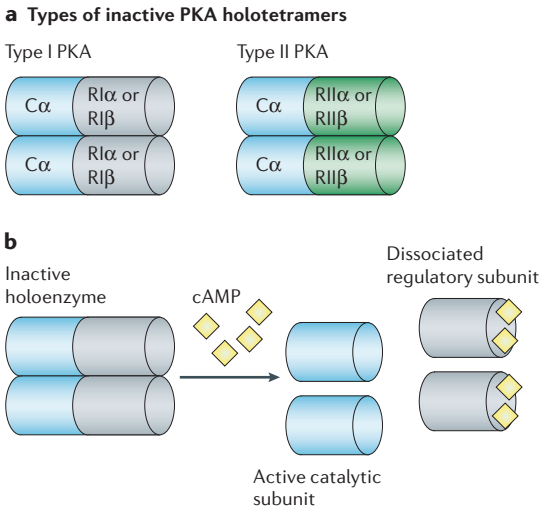

Figure 2 | **Schematic diagram of PKA structure and activation. a** | Schematic of the inactive protein kinase A (PKA) holoenzyme depicts the type I holotetramer, which is comprised of two catalytic subunits associated with either RIα or RIβ regulatory subunits, and the type II holotetramer, which is comprised of two catalytic subunits associated with either RIIα or RIIβ regulatory subunits. **b** | Schematic illustration of cyclic AMP (cAMP)-dependent activation of the PKA holotetramer. Two cAMP molecules bind to each regulatory subunit within an inactive holotetramer. The binding produces a conformation change, and the regulatory subunits dissociate from the catalytic units. The liberated catalytic units are enzymatically active and can phosphorylate downstream targets.

Further linkage analyses mapped the disease gene in this CNC–TPS family to chromosome 17p12–13.1 (REF. 9), and a subsequent positional-candidate cloning strategy showed a missense mutation (R674Q) in *MYH8*, which encodes the perinatal isoform of myosin heavy chain[8]. The same founder mutation was identified in other families with reportedly isolated TPS, but subsequent detailed family histories have indicated that members of these families are also potential CNC patients.

The observation that a mutation in a structural protein, especially one not known to be phosphorylated by PKA, can cause CNC was surprising, and the mechanism through which the *MYH8* mutation participates in the formation of cardiac myxomas remains unknown. Perinatal myosin is expressed in myofibroblasts, and it has been suggested that cardiac myxomas might arise from poorly characterized pluripotent sub-endocardial stem cells[7,12–15]. Given this, it is possible that genetic abnormalities in perinatal myosin might promote the survival of cardiac progenitor cells such that these cells remain within the diverse myofibroblast population in adult life. Because they retain the ability to proliferate they could undergo further tumorigenic genetic events[9]. How such events potentially intersect with abnormal PKA signalling awaits a better understanding of PKA's contribution to cardiac tumorigenesis.

However, *MYH8* and *PRKAR1A* are certainly not the only genes that cause CNC and cardiac myxomas when mutated. These studies have identified families in which CNC is not associated with mutations at either of these loci or the hypothesized chromosome 2p locus. Therefore, linkage studies to establish additional genetic loci for CNC are ongoing.

Regulation and function of PKA

A key question for ongoing investigation, then, is the mechanism by which haploinsufficient loss of R1α leads to CNC phenotypes and tumorigenesis. PKA is a prototypical mediator of cAMP transduction in mammalian cells[40] and its subunits have multiple isoforms that can confer specific activities, cAMP-binding affinities, expression patterns and different subcellular localization. Independent genes[52–55] encode three catalytic subunit isoforms (*PRKCA*, *PRKCB* and *PRKCG*, which encode Cα, Cβ, and Cγ proteins, respectively) and four regulatory subunits (*PRKAR1A*, *PRKAR1B*, *PRKAR2A* and *PRKAR2B*, which encode RIα, RIβ, RIIα and RIIβ proteins, respectively)[56–58]. Combinations of these regulatory and catalytic subunits result in molecular complexes that are categorized as either type I or type II holoenzymes, depending on the type of regulatory subunit that is used — RIα/β versus RIIα/β[30,38,59] (FIG. 2).

Most cells express some form of both type I and type II PKA, but the level of expression and the ratio of type I to type II differs significantly between tissues[60,61]. Moreover, the level of expression of specific regulatory subunit genes varies from cell type to cell type and throughout development, even within a given cell type. Whereas α-type regulatory subunits are expressed in nearly all tissues, the β subunits are enriched in selected tissues — brain, adipose and testicular tissue[60,61]. Regulating the expression and utilization of PKA regulatory subunits has the potential to confer unique patterns of subcellular localization and distribution through the interaction of regulatory subunits with various A-kinase-anchoring proteins (AKAPs)[62,63]. Type I PKA is usually distributed diffusely in the cytoplasm[41,64] but can be redirected to specific intracellular sites, such as the microtubules, during cell division[65]. Type II PKA is commonly localized to intracellular compartments[59,66] within the membrane soluble fraction.

AKAPs were initially identified by their ability to bind to the RII subunits of PKA[67,68]. More recently however, dual AKAPs have been described that bind both RI and RII subunits[69]. So far, more than 70 different AKAPs have been discovered in a range of cell types, and each AKAP shows specificity to various organelles, the plasma membrane and neuromuscular junctions[70–72]. AKAPs also function as scaffolds to bind other signalling proteins[73,74], and in doing so synchronize and integrate PKA signalling at the target site, producing a high degree of specificity and control. As a result, the interaction of the regulatory subunit of PKA with AKAPs is vital in modifying the response of PKA that is mediated by cAMP. Monospecific AKAPs that interact only with type I regulatory subunits have yet to be identified.

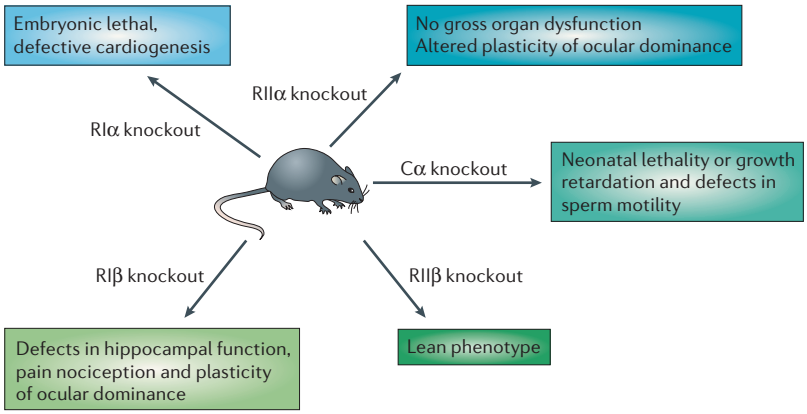

Figure 3 | **Genetically engineered mouse models to study the function of each PKA subunit.** Knockout mouse models have been generated for many protein kinase A (PKA) subunits. These models have varying phenotypes (indicated in the figure) that have shed light on some aspects of PKA function.

Lessons from mouse models

With the use of *in vivo* and *in vitro* techniques, the function of each component of the PKA holoenzyme has been studied extensively. Phenotypic analyses of knockout mice have also shed light on how adult and embryonic cells respond to and compensate for the loss of each PKA holoenzyme component (FIG. 3).

Prkca-null mice[75] typically die shortly after birth. However, some animals survive to adulthood. These mice demonstrate growth retardation and defects in sperm motility. Many tissues that are examined from Cα-knockout mice show a significant decrease in cAMP-stimulated PKA kinase activity — <10% of normal PKA activity. The loss of the Cα subunit also results in a dramatic reduction of both RIα and RIIα protein subunit expression levels. The regulatory subunits are stabilized when they are bound to the catalytic subunits. Therefore, the reduction in regulatory subunit expression levels is probably a consequence of increased degradation in the setting of decreased amounts of Cα. The absence of complete lethality in *Prkca*-null mice implies that, at least in surviving animals, compensatory mechanisms must exist to counterbalance the loss of this important catalytic subunit of PKA.

Unlike Cα-knockout mice, mice with genetically engineered deletions of PKA regulatory subunits have non-lethal phenotypes. For instance, *Prkar1b*-null mice[76] show normal survival, but do have severe defects in hippocampal function as well as other neurological processes. Whereas a compensatory increase in levels of RIα protein expression occurs in these mice, there are no detectable changes in PKA activity in the tissues that have been analysed. *Prkar2b*-null mice[77] also survive to adulthood, but show a lean phenotype. RIIβ protein is abundantly expressed in white and brown adipose tissue as well as in the brain, and *Prkar2b*-null brown adipose tissue has increased levels of RIα protein expression and increased PKA activity. It is thought that this results in an increased metabolic rate with a consequent lean phenotype. Expression of *Prkar2b* in the brain might also be crucial, given the established roles for this protein in neurological processes such as motor function and sensitivity to drug abuse. *Prkar2a*-null mice[78] have no grossly evident phenotype (except for altered plasticity of ocular dominance[79]) but the loss of RIIα is associated with altered expression of other PKA subunits. There is a compensatory increase in RIα protein as well as a decrease in Cα protein. The net result is decreased PKA activity in tissues such as adult skeletal muscle. The compensatory changes in other regulatory subunits could account for the largely normal phenotype of *Prkar2a*-null mice.

Consistent with its role in the pathogenesis of human disease, complete loss of RIα has a profound effect on mouse structure and function[80], and the striking phenotype of *Prkar1a*$^{-/-}$ mice illustrates the importance that this particular subunit has in regulating PKA activity in early embryonic development. *Prkar1a*-null mice show high basal levels of PKA activity and have marked developmental defects[80]. They show growth retardation and severe maturation and migration defects in mesoderm-derived tissues, including the heart. The mice die *in utero* at embryonic day E10.5, and no compensatory increase in the expression levels of type II subunits is observed. Fibroblasts cultured from these mice seem to be spontaneously immortalized (P. Amieux, personal communication). Embryonic lethality can be partially rescued by crossing into a *Prkaca*$^{+/-}$ background. *Prkar1a*$^{-/-}$/*Prkaca*$^{+/-}$ embryos have decreased basal levels of PKA activity. With further loss of Cα by crossing into a *Prkaca*$^{-/-}$ background, the rescue is even more complete. Such embryos show an even greater decrease in basal PKA activity as well as increased somite number and more normal development of limbuds and head mesenchyme, and a more mature heart tube than their *Prkar1a*$^{-/-}$/*Prkaca*$^{+/-}$ counterparts. Therefore PKA activity as regulated by R1α is required for normal murine embryogenesis.

PRKAR1A contribution of to CNC pathogenesis

We[8] and others[35] initially suggested that *PRKAR1A* functions as a classic tumour-suppressor gene in CNC. This hypothesis was based on observations that showed that most reported *PRKAR1A* mutations resulted in haploinsufficiency. In addition, several studies have demonstrated that some of the tumours isolated from patients with CNC show LOH at the *PRKAR1A* locus[35,49]. For instance, analysis of adrenocortical tumours[81], a tissue that is frequently associated with dysplasia in patients with CNC, showed that a significant number of tumours display *PRKAR1A* LOH. However, *PRKAR1A* LOH is not present in all tumours[82], and it is remarkable that when LOH does occur, it might be secondary to DNA instability in tumours rather than a primary cause of tumorigenesis. It is well recognized that chromosome 17 is the chromosome most commonly found to be rearranged in human tumours[83]. So, it is important to determine whether *PRKAR1A* LOH is required or even necessary for tumour formation.

The ability of *Prkar1a* haploinsufficiency to induce tumours in the absence of *Prkar1a* LOH was highlighted by an investigation in *Prkar1a*-knockout mice. *Prkar1a*$^{-/+}$ mice appear grossly normal[80], but closer examination demonstrates that these animals show several features

Plasticity of ocular dominance
Similar to handedness, people usually have a dominant right or left eye — this is referred to as ocular dominance. In some circumstances, this can be modified by genetic and/or environmental factors (plasticity).

in common with CNC patients[8]. For instance, both *Prkar1a*$^{-/+}$ mice and patients with CNC show defects in sperm maturation with consequent impairment of male fertility. Importantly, this is dependent on the genetic background of the mice; most *Prkar1a*$^{-/+}$ C57BL/6 mice are infertile, whereas *Prkar1a*$^{-/+}$ SvJ/129 mice are not. In addition, electrocardiographical analyses show marked suppression of heart-rate variability in the mice as well as the patients[8]. In humans, such decreased heart-rate variability has been associated with sudden death[84–86], which is occasionally seen in patients with CNC.

In addition, *Prkar1a*$^{-/+}$ mice, similar to patients with CNC, show an increased susceptibility to tumour formation as they age. A similar age-dependency was observed by Griffen *et al.*[87] in transgenic mice in which *Prkar1a* translation was variably reduced by a constitutively expressed *Prkar1a* antisense construct. In *Prkar1a*$^{-/+}$ mice, a wide range of tumours are seen that generally fall under the category of sarcomas. Although such malignant tumours are not thought to be features of CNC, the murine tumours often include myxomatous regions that are reminiscent of the myxomas in patients with CNC. In addition, a low but increasing number of CNC patients with malignant sarcomas have been detected (D. A. McDermott and C.T.B., unpublished data). The most common tumours in *Prkar1a*$^{-/+}$ mice are haemangiosarcomas of the spleen, soft tissue sarcomas in skeletal muscle and chondrosarcomas of the axial skeleton. In a similar haploinsufficient mouse, Kirschner *et al.*[88] have also observed endocrine tumours. Studies are ongoing to investigate the potential effects of genetic background (as in the case of male infertility) on tumorigenesis, and the capacity of *Prkar1a*$^{-/+}$ mice to mimic CNC tumorigenesis. However, regardless of tumour type, analysis of tumour tissue from *Prkar1a*$^{-/+}$ mice fails to demonstrate convincing and consistent *Prkar1a* LOH. As demonstrated by both western blot and immunohistochemistry, the tumour cells that arise in *Prkar1a*$^{-/+}$ mice continue to express wild-type R1α protein. So, tumorigenesis in these mice does not require complete loss of *Prkar1a*, and *Prkar1a* haploinsufficiency is adequate to predispose to tumorigenesis.

An important caveat to all such experimental studies is the recent observation that a missense mutation (R74C) of *PRKAR1A* can also produce tumours in human patients with CNC[8]. Current data does not demonstrate any deficiency in regulation of the PKA holotetramer by R74C-R1α[8]; cells that are transfected with either wild-type R1α or R74C-R1α show indistinguishable reductions in cAMP-stimulated PKA activity. Therefore, other currently unknown mechanisms, aside from *PRKAR1A* haploinsufficiency, might be operating in *PRKAR1A*-related CNC.

In addition, studies to date do not exclude a contribution of *Prkar1a* LOH to some specific tumour types. Kirschner *et al.*[88] have recently conditionally deleted one *Prkar1a* allele in the murine schwann cell compartment and demonstrated that this genetic loss results in schwannoma formation. In these tumours, fluorescent *in situ* hybridization (FISH) analysis with a *Prkar1a* mouse bacterial artificial chromosome (BAC) clone as a probe failed to consistently demonstrate two *Prkar1a* alleles in all tumour cells. Approximately one-third to one-half of tumour cells showed a signal for only one allele, and Kirschner *et al.*[88] have suggested that perhaps these cells have *Prkar1a* LOH and promote tumorigenesis by a paracrine effect on those cells without *Prkar1a* LOH. However, the absence of two FISH signals might still represent technical limitations in the consistency of the assay, and these investigators did not perform immunohistochemistry to determine the continued presence or absence of R1α protein. Furthermore, it remains unclear if variable *Prkar1a* LOH in an established tumour is not a reflection of the stochastic consequences of genomic instability that occur after tumour formation rather than true tumorigenic events. Strikingly, none of these mouse models have yet been able to address the specific requirement for *Prkar1a* LOH during cardiac tumorigenesis as they have not replicated the cardiac myxomas that are seen as a cardinal feature of human CNC.

Investigation of the male infertility phenotype in *Prkar1a*$^{+/-}$ mice has indicated that altered PKA activity might contribute to its pathogenesis, as crossing these mice with *Prkaca*$^{+/-}$ mice reduces PKA activity and results in normal male fertility (K. Burton, personal communication). A potential contribution of altered PKA activity to CNC tumorigenesis is supported by the observation that some CNC adrenal tumour cells show higher levels of cAMP-stimulated PKA activities than non-CNC adrenal tumour cells[89]. However, analyses of tumour tissue in *Prkar1a*$^{-/+}$ mice raise questions regarding the role of altered PKA activity in CNC tumorigenesis. Basal levels of PKA activity in tumour tissue are low but largely similar to the PKA activities of surrounding non-neoplastic tissue. Still, the relationship of cAMP-stimulated levels of PKA activity in tumour tissue to those in normal surrounding tissue is less clear and highly variable. Increased, decreased and unchanged activities of cAMP-stimulated PKA are all seen in tumour tissue[8], and this variability indicates that altered PKA activity in tumours might be a consequence of increased genomic instability after tumorigenesis, rather than being a primary facilitator of tumorigenesis.

In several tissue types, including Schwann cells, increased mitogenesis is associated with increased or unregulated PKA activity. However, in other cell types, including mesodermally derived cells such as vascular smooth muscle cells, increased PKA activity inhibits mitogenesis. This inconsistency between different cell types highlights the tissue-specific manner in which PKA regulates cell proliferation, and the complexity of predicting the consequence of alterations in PKA activity or in concluding pathogenicty of altered PKA activity. Studies that address specific intracellular-microdomain-PKA activities, as opposed to total-cellular or tissue PKA activity, have yet to be performed. Such experiments might yet identify crucial changes in PKA activities within these subcellular pools that will provide consistent mechanisms of pathogenesis.

PKA isoforms in CNC and non-CNC tumours

In vivo and *in vitro* studies have shown that R1α can function as a mediator of either mitogenic stimuli or proliferation repressors depending on the cell type under study. Crucial to this is the ratio of type I PKA to type II PKA. Analyses of type I and II regulatory subunits in mouse models as well as in CNC tumours show that expression of these subunits can be coordinately regulated in normal physiological environments as well as in pathological states. As described above, genetic deletion in the mouse of a type I regulatory subunit often leads to compensatory upregulation of type II regulatory subunits. However, Casey *et al.*[34] demonstrated that, unlike non-neoplastic cells from patients with CNC in which R1α remains in significant excess over R2β, CNC cardiac myxoma cells show a reversal of this ratio with an excess of R2β over R1α. The functional contribution to tumorigenesis of such PKA isoform switching remains to be determined.

Studies indicate that the coordinated regulation of PKA isoforms also has a pivotal function in non-CNC tumour models by modulating the phosphorylation of various transcription factors such as CREB and nuclear factor-κB (NF-κB) in addition to tumour-suppressor proteins such as LKB1 (also known as Ser/Thr protein kinase 11) (REFS 90–92). In pituitary adenomas, which are benign neoplasms that are similar to the lesions seen in CNC, low levels of R1α expression combine with high levels of R2α and R2β expression to unbalance the ratio of type I to type II PKA and thereby promote increased cell proliferation[93]. However, malignant tumours could use type I and type II PKA signalling in a different fashion. Unlike the benign tumours of CNC in which R1α expression is decreased, specific increases in R1α protein expression levels and type I PKA expression levels has been observed in several human malignant tumours such as breast, colon, ovarian and renal-cell carcinoma[94–98]. Such increased expression of type I PKA correlates with active malignant cell growth, increased proliferation and poor patient prognosis[96,99–101].

Investigators have therefore focused on R1α as a potential target for anticancer therapy in these non-CNC malignant tumours. Cho-Chung and colleagues[102] used antisense R1α compounds to obtain sustained inhibition of human colon carcinoma growth in athymic mice. In these studies, single injections of antisense *PRKAR1A* RNA reduced R1α expression levels in conjunction with compensatory increases in R2β protein expression levels. Additional experiments with *PRKAR1A* antisense RNA treatment in malignant colon, prostate and pancreatic tumour cell lines have demonstrated inhibition of cell growth, blocking of cell proliferation and induction of apoptosis[103–109]. So, depending on the cell or tissue type, R1α can be pro-tumorigenic or anti-tumorigenic, and it will be important for future studies to define the role of R1α and PKA-signalling in specific tissue and cell-biological contexts.

Conclusion and future directions

R1α is a key component in regulating the cAMP-dependent PKA enzyme and has a pivotal role in modifying signal transduction in a wide variety of intracellular pathways. Studies of *PRKAR1A* mutations in CNC have supported a role for *PRKAR1A* haploinsufficiency in CNC tumorigenesis, but ongoing questions about the contributions of *PRKAR1A* LOH, potential changes in PKA activity and secondary changes in expression of other PKA regulatory subunit genes prompt consideration of alternative or additional *PRKAR1A* pathways to tumorigenesis. Moreover, evidence of pathogenic missense mutation of *PRKAR1A* in the absence of haploinsufficiency highlights the need for investigation of PKA-dependent mechanisms of tumorigenesis. Future studies will also need to incorporate other genes that can be mutated to produce CNC and cardiac tumorigenesis. The recently characterized MYH8 mutation is the first identification of one of these additional genes, but it seems certain that other causative genes remain to be identified. Ultimately, the ability to establish molecular genetic cascades that regulate cardiac cell growth will have significant implications not only for tumour biology and treatment, but also for creative new approaches to promote regenerative cardiac cell growth to tackle cardiomyopathies and ischaemic heart disease.

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Competing interests statement
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