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α-Methylacyl-CoA racemase – an 'obscure' metabolic enzyme takes centre stage

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Branched-chain lipids are important components of the human diet and are used as drug molecules, e.g. ibuprofen. Owing to the presence of methyl groups on their carbon chains, they cannot be metabolized in mitochondria, and instead are processed and degraded in peroxisomes. Several different oxidative degradation pathways for these lipids are known, including α-oxidation, β-oxidation, and ω-oxidation. Dietary branched-chain lipids (especially phytanic acid) have attracted much attention in recent years, due to their link with prostate, breast, colon and other cancers as well as their role in neurological disease. A central role in all the metabolic pathways is played by α-methylacyl-CoA racemase (AMACR), which regulates metabolism of these lipids and drugs. AMACR catalyses the chiral inversion of a diverse number of 2-methyl acids (as their CoA esters), and regulates the entry of branched-chain lipids into the peroxisomal and mitochondrial β-oxidation pathways. This review brings together advances in the different disciplines, and considers new research in both the metabolism of branched-chain lipids and their role in cancer, with particular emphasis on the crucial role played by AMACR. These recent advances enable new preventative and treatment strategies for cancer.

Introduction

Branched-chain fatty acids and related compounds are important components of the human diet and are also used as drug molecules. Owing to the presence of methyl groups on the carbon chain, the majority cannot be immediately metabolized within mitochondria, and instead undergo initial metabolism in peroxisomes [1–4]. A consequence of the presence of methyl groups on the carbon chain is that many of these fatty acids contain chiral centres. Methyl groups can be located on both the two and three carbon positions, and this has consequences for metabolism. The oxidation of these fats is stereoselective [1], and this has consequences for the regulation of metabolism.

Branched-chain fatty acids can arise from several different sources. Humans endogenously synthesize bile acids, which are oxidized cholesterol derivatives. These acids possess the methyl group on carbon 2 (relative to the carboxyl group), and have exclusively (*R*)-stereochemistry. In terms of quantity, non-steroidal fatty acids are the most important. Pristanic acid is a minor component of the diet, and it possesses four methyl groups [1–4]. The methyl group at C-2 can have either the (*R*)-configuration or (*S*)-configuration, whereas the other methyl groups have exclusively the (*R*)-configuration.

Abbreviations
ACOX, acyl-CoA oxidase; AMACR, α-methylacyl-CoA racemase; CYP, cytochome P450; FALDH, fatty aldehyde dehydrogenase; FAR and MCR, α-methylacyl-CoA racemase from *Mycobacterium tuberculosis*; PhyH, phytanoyl-CoA 2-hydroxylase; PPAR, peroxisome proliferation-activated receptor.

Phytanic acid is its 3-methyl dietary precursor, with stereochemistry identical to that of pristanic acid. Phytanic acid is originally derived from the isoprenoid side-chain of chlorophyll A, phytol, although it is generally believed that phytol cannot be cleaved from chlorophyll in plant-derived foods and that phytanic acid comes directly from animal products. Foods that are particularly rich in phytanic acid include beef, other meats and dairy products. A typical daily intake of phytanic acid in a Western diet has been estimated to be 50–100 mg [2]. Finally, anti-inflammatory drugs such as ibuprofen are 2-methyl acids [1]. These drugs differ in that they have short, branched carbon chains attached to an aromatic moiety.

Much of the metabolism of branched-chain lipids takes place in peroxisomes [1–6], and has been studied since the 1960s. Peroxisomes are ubiquitous organelles found in virtually all eukaryotic cell types [7], and are responsible for the synthesis of essential fatty acids (such as ether phospholipids) and detoxification of 'unusual' fatty acids and related lipids (ultra- and very-long-chain fatty acids, branched-chain fatty acids, etc.) [1]. Deficiency of peroxisomes or their key metabolic pathways gives rise to the peroxisomal biogenesis disorders [8], such as Zellwegers' syndrome and infantile Refsum's disease. Milder syndromes can result from single-enzyme [9] deficiencies in preliminary pathways (especially α-oxidation [10]; see below), and give rise to neurological diseases such as adult Refsum's disease and racemase deficiency [2]. These conditions were considered to be biochemical oddities, due to the low number of patients affected.

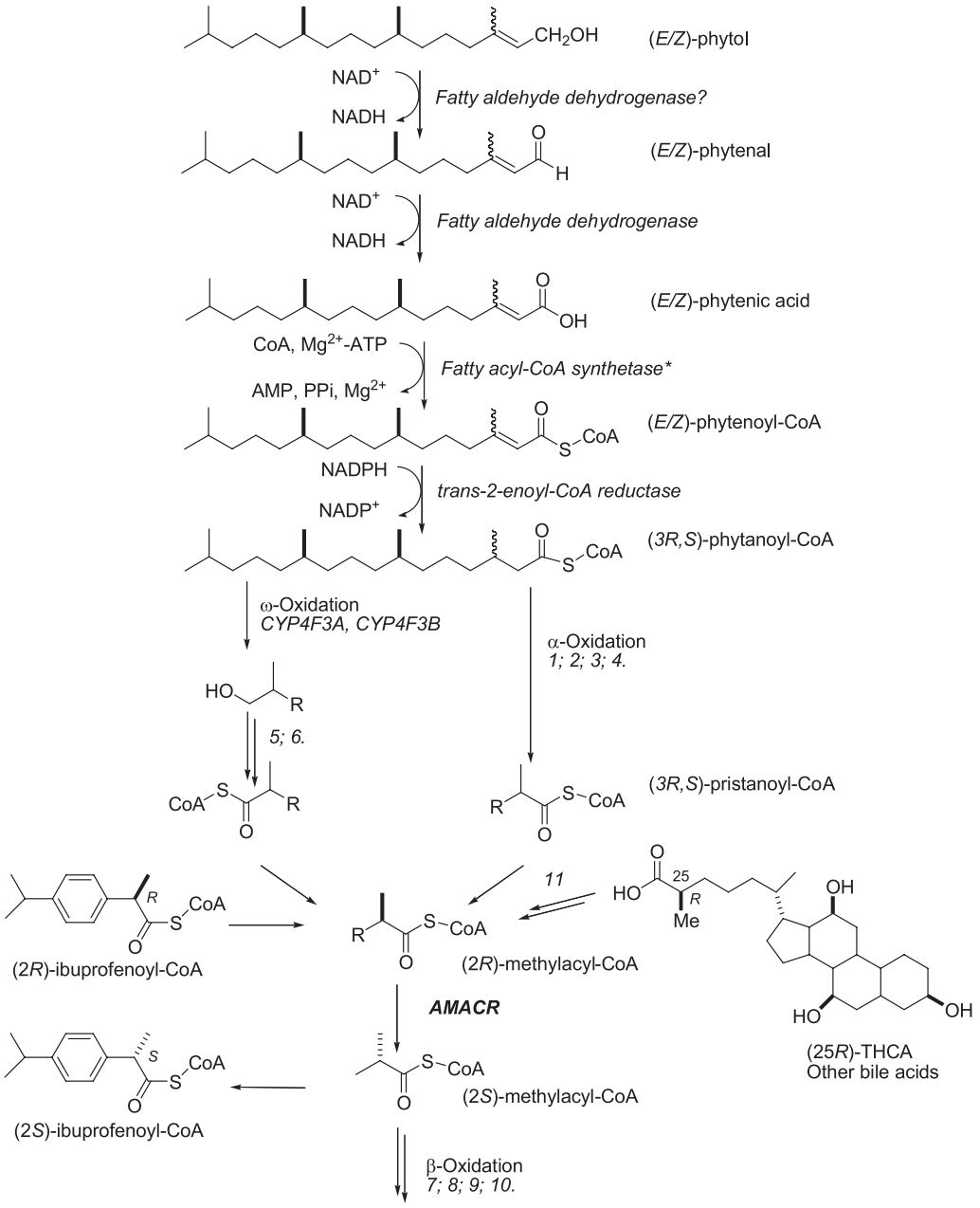
Since 2001, it has become apparent that there is a link between dietary branched-chain fatty acids (phytanic acid), activity of the metabolic pathways, and disease, with a particularly strong correlation with prostate cancer [11,12]. This review will look at recent progress in understanding branched-chain fatty acid metabolism and its link with cancer. One particular enzyme, α-methylacyl-CoA racemase (EC 5.1.99.4) (AMACR, racemase, P504S), has emerged as a cancer marker, and the central biochemical role of this enzyme is discussed.

Branched-chain fatty acid metabolism

The α-oxidation pathway for phytanic acid (and presumably other 3-methyl acids) was finally elucidated about 10 years ago [1–4]. Significant further progress has been made, including considerable advances in understanding the conversion of free phytol to phytenoyl-CoA, which can be converted to pristanic acid. Further progress has also been made in understanding ω-oxidation, a secondary degradation pathway for phytanic acid (Scheme 1). The presence of 3-methyl groups in phytanic acid prevents β-oxidation, as a quaternary alcohol is produced from this substrate. Hence, phytanic acid undergoes preliminary α-oxidation, in which chain shortening from the carboxyl group occurs. This pathway produces pristanic acid, which has a 2-methyl group, and hence β-oxidation is not blocked.

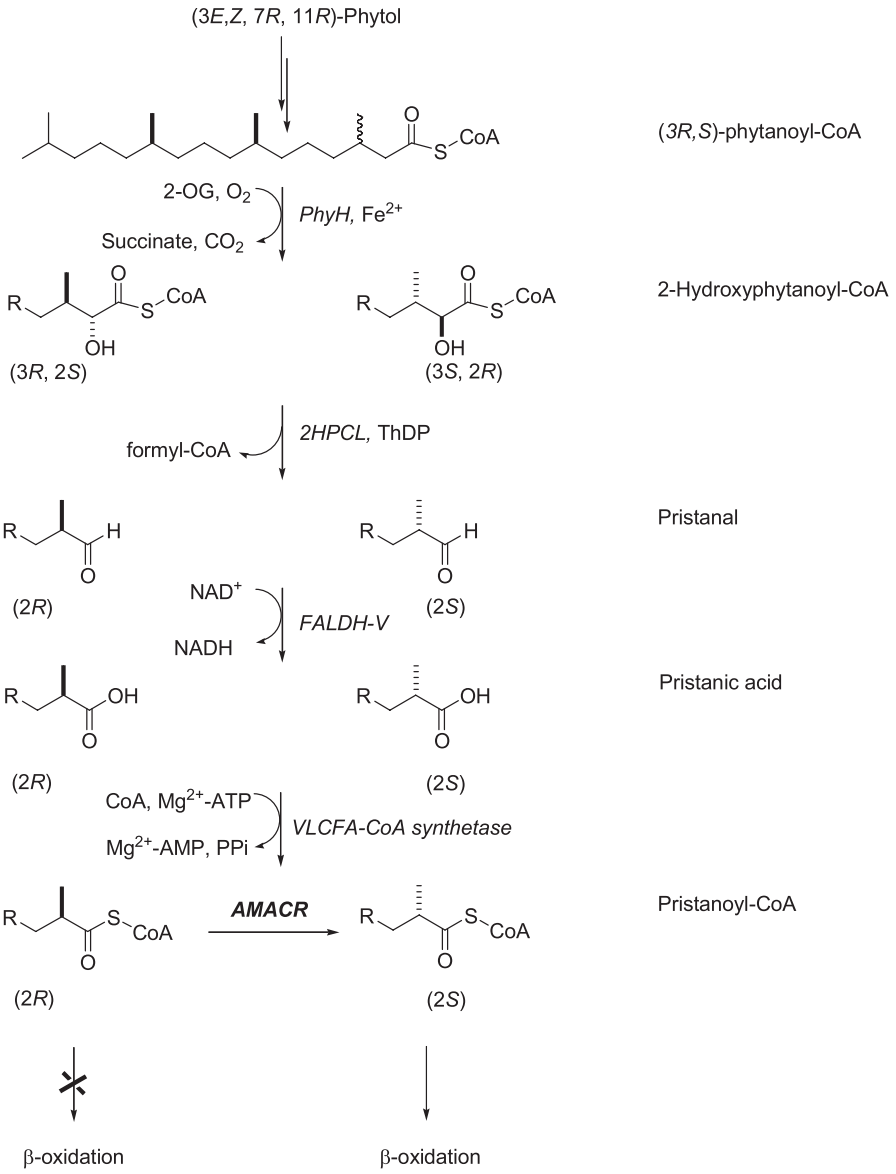
The α-oxidation pathway consists of four steps [1], the first being conversion of phytanic acid to its CoA ester and peroxisomal import (Scheme 2). This is followed by hydroxylation by a nonhaem iron(II) and a 2-oxoglutarate-dependent oxygenase, phytanoyl-CoA 2-hydroxylase (PhyH). Adult Refsum's disease is a result of inactivating mutations in this enzyme [13,14] or of defects in the system responsible for importing this protein into peroxisomes [15]. The X-ray crystal structure of PhyH has recently been solved [13], and this demonstrates that the majority of clinical mutations cluster around the iron(II) cofactor- or 2-oxoglutarate cosubstrate-binding sites. Site-directed mutagenesis studies have demonstrated the functional importance of the iron(II)- and 2-oxoglutarate-binding ligands [14,16,17]. In common with many other nonhaem iron(II)-dependent oxygenases [18], PhyH is able to accept unnatural substrates [19] with 3-methyl or other alkyl groups, but is not able to accept substrates with alkyl groups at either C-2 or C-4. The product of the PhyH-catalysed reaction, 2-hydroxyphytanoyl-CoA, is cleaved to pristanal and formyl-CoA, and the latter is subsequently converted to formate and then to CO_2 [1–3]. This unusual thiamine diphosphate-dependent lyase has also been implicated in the degradation of unbranched straight-chain 2-hydroxy acids [20]. Finally, pristanal is oxidized to pristanic acid, which is converted to pristanoyl-CoA [1–3]. There is also evidence for the involvement of the fatty acid-binding protein, sterol carrier protein-2, in at least some steps of both α-oxidation [1,21] and β-oxidation (as sterol carrier protein-x) [1].

Recently, it has been demonstrated that the phytol side-chain of chlorophyll A can be converted into phytanic acid by humans [22–27]. The pathway consists of oxidation of the allylic alcohol to the highly reactive aldehyde, phytenal, followed by further oxidation to phytenic acid (Scheme 1). The enzyme performing the phytenal-to-phytenic acid conversion was identified as fatty aldehyde dehydrogenase (FALDH) [25], the enzyme that is deficient in Sjögren–Larsson syndrome [1,28]. Studies on recombinant FALDH showed that it was also able to oxidize alcohols to aldehydes [29].



Scheme 1. Metabolism of branched-chain fatty acids and related compounds. *Peroxisomes contain more than one fatty acyl-CoA synthetase, and it is not clear which specific enzyme is responsible for the phytenic acid-to-phytenoyl-CoA conversion. Enzymes, cosubstrates and cofactors [1,2]: 1, phytanoyl-CoA 2-hydroxylase, iron(II), 2-oxoglutarate, O_2; 2, 2-hydroxyphytanoyl-CoA lyase (also known as 2-hydroxyacyl-CoA lyase), Mg^{2+}-thiamine diphosphate; 3, FALDH-V, CYPs; 4, very-long-chain fatty acyl-CoA synthetase, Mg^{2+}-ATP, CoA-SH; 5, 6, unidentified oxidoreductases or CYP enzyme – Reactions will go via aldehydes and acid intermediates; 7, branched-chain acyl-CoA oxidase, FAD; 8, 9, *D*-bifunctional protein, NAD^+; 10, sterol carrier protein-x (SCP-x), CoA-SH; THCA, trihydroxycholestanic acid.

Although conversion of phytol was not demonstrated, the use of a bifunctional oxidoreductase would prevent the release of the highly reactive allylic aldehyde, phytenal. Phytenic acid is converted to its CoA ester and reduced to phytanic acid by an NADPH-dependent oxidoreductase [26,30]. It is not clear how much plant-derived phytol is converted into phytanic acid in humans, as humans are not supposed to be able to cleave this side-chain from chlorophyll, although some contribution from gut bacteria cannot be excluded



Scheme 2. α-Oxidation of (3*R,S*)-phytanoyl-CoA. Both epimers of phytanoyl-CoA can undergo α-oxidation; the (2*R*)-epimer of pristanoyl-CoA is converted to (2*S*)-pristanoyl-CoA by AMACR for β-oxidation. 2-HPCL, 2-hydroxyphytanoyl-CoA lyase (also known as 2-hydroxyacyl-CoA lyase); 2-OG, 2-oxoglutarate; THDP, thiamine diphosphate; VCLA-CoA synthetase, very-long-chain fatty acyl-CoA synthetase.

[11,31]. A recent epidemiological study showed that plasma phytanic acid levels were strongly correlated with dairy fat intake [32] but not vegetable intake, suggesting that the amounts directly derived from chlorophyll are relatively small.

The α-oxidation pathway was defined about 10 years ago [1–3,10], and consists of formation of the phytanoyl-CoA ester followed by 2-hydroxylation, an unusual lyase reaction giving pristanal, and finally oxidation of pristanal to pristanic acid (Scheme 2). All of the enzymes catalysing these steps were defined at this time, except for the enzyme performing the pristanal-to-pristanic acid conversion. It was proposed that oxidation of the aldehyde function of pristanal was performed by FALDH [33], but later experiments cast doubt on this, on the grounds that significant residual 'pristanal dehydrogenase' activity was observed in FALDH-deficient cells [34]. Moreover, the major form of FALDH (FALDH-N) is localized in the endoplasmic reticulum [35,36], and α-oxidation is known to be exclusively peroxisomal [34]. A second splice variant of

FALDH [37] has been identified (FALDH-V), and very recently it has been shown to localize in peroxisomal membranes [38]. Two further splice variants (FALDH-V2 and FALDH-V3) were also identified [38], although these appear not to be localized in peroxisomes. The authors propose that FALDH-V catalyses the conversion of pristanal to pristanic acid, and this is supported by the observation that overexpression of FALDH-V but not FALDH-N protects cells against phytanic acid-induced damage. Production of all four protein splice variants of FALDH are induced by peroxisome proliferation-activated receptor (PPAR)α agonists, and increased expression of FALDH-N and FALDH-V protects against lipid peroxidation. The low level of residual pristanal dehydrogenase activity in Sjogren–Larsson syndrome fibroblasts was attributed to incomplete loss of activity in FALDH mutants [34,38]. However, PPARα agonists were also shown to induce several other genes in addition to *aldh3a2* (the gene encoding for the FALDH splice variants), including several cytochome P450 (CYP) enzymes [39]. It could be that one or more CYP enzymes play a secondary role in the pristanal-to-pristanic acid conversion.

Although α-oxidation is the primary metabolic pathway for phytanic acid, some metabolism can also occur by ω-oxidation [40–44]. Clinically, ω-oxidation is important in patients deficient in α-oxidation, such as those suffering from adult Refsum's disease [40], as it provides a route by which phytanic acid can be detoxified. The process requires hydroxylation by a CYP hydroxylase followed by conversion of the alcohol into the acid, and is probably localized in microsomes (Scheme 1). In the case of phytanic acid, the specific hydroxylases have been identified as CYP4F3A and CYP4F3B, with lower activity for CYP4F2 and CYP4A11 [43]. The ω-oxidation pathway generates a new chiral centre in the molecule as a 2-methyl acid (relative to the new carboxyl group), for which the stereochemistry has not been determined [40]. The resulting di-acids can be exported to peroxisomes for subsequent β-oxidation as the CoA ester [40]. This process could potentially allow a large number of substrates to enter into peroxisomal β-oxidation, and this pathway is known to be active in the production and metabolism of bile acids from cholesterol [45].

Peroxisomes contain two β-oxidation pathways, and it is the pathway whose genes are constitutively expressed that metabolizes branched-chain fatty acids [1]. This pathway only metabolizes fatty acids with (2*S*)-stereochemistry [46], as their CoA esters. Bile acids are exclusively produced with (2*R*)-stereochemistry [47,48], and as (2*R*)-methyl groups are encountered during the degradation of pristanic acid and its precursors, chiral inversion is required. This process is achieved by AMACR [1], a reversible enzyme that interconverts the two epimers, and therefore controls entry into the β-oxidation pathway. The β-oxidation pathway chain shortens the fatty acids by two carbons during each cycle. In the case of pristanic acid, β-oxidized fragments, such as acetyl-CoA and propionoyl-CoA, and chain-shortened intermediates are exported into mitochondria for final metabolism via the acyl-carnitine shuttle [1]. As these chain-shortened intermediates also contain chiral methyl groups with the (*R*)-configuration, AMACR is also required within mitochondria (see below) for β-oxidation to occur. It is not known whether chain-shortened bile acids are similarly exported to the mitochondria. Patients deficient in AMACR exhibit neurological symptoms [49] with some similarities to adult Refsum's disease [2] but with later onset and a more peripheral than central neurological phenotype. They exhibit the expected biochemical profile, with accumulation of bile acids and dietary (2*R*)-branched acids [47,50]. A 'knockout' mouse model is also available, and this shows a similar metabolic profile, with upregulation of expression for several genes, including those encoding CYP enzymes that may be involved in ω-oxidation [51].

Ibuprofen is a 2-methyl acid, and is generally given as a racemic mixture of (2*R*)- and (2*S*)-enantiomers. Activation as the CoA ester and chiral inversion [52–56] have been implicated in both pharmacological activity and toxic side-effects. The enzyme responsible for this is 'ibuprofenoyl-CoA epimerase' [52], which, upon cloning, proved to be identical to AMACR [57,58]. AMACR is able to utilize both (2*R*)- and (2*S*)-ibuprofenoyl-CoA as substrates [52]. Formation of the CoA ester has been reported to be stereoselective for the (2*R*)-isomer, whereas hydrolysis of both isomers can occur [52,59], implying that the physiological process is the (2*R*) to (2*S*) conversion, i.e. the same as that for fatty acid metabolism. Ibuprofen is an aromatic structure substituted with a 2-methyl acid, and cannot undergo β-oxidation.

Branched-chain fatty acids and cancer

In 2001, several reports appeared in the literature showing that AMACR protein was overproduced in various cancers [60]. Since then, more than 280 reports have appeared in the literature documenting overproduction of AMACR in cancer [61]. The majority of

reports have focused on prostate cancer [11,12,62–64], as the levels of overproduction are high (up to ninefold higher than in noncancerous cells [65]) and consistently observed [11]. This level of overproduction has led to the use of antibody-based methods to diagnose prostate cancer from biopsy samples, with the marker known as P504S [60]. Zha *et al.* [66] demonstrated that AMACR is an androgen-independent growth modifier in prostate cancer cells. AMACR is also overproduced in some noncancerous prostatic abnormal states [67] and neoplasia [68]. Although most of the reports on the overproduction of AMACR concern prostate cancer, other studies have shown that overproduction can also occur in breast [69], colon [63], renal [70,71] and other cancers [61,72], although there is considerable heterogeneity in the degree of overproduction (for example, Jiang *et al.* [61] reported that only 27% of gastric adenocarcinomas overproduce AMACR).

Since then, a large body of evidence has linked dietary branched-chain lipid intake (especially phytanic acid), AMACR overproduction [11,12], and cancer. Xu *et al.* [73] reported that dietary phytanic acid intake and levels in the blood directly correlate with prostate cancer risk, whereas Mobley *et al.* [74] showed that dietary branched-chain fatty acids increased production of AMACR in prostate cancer cells, with catalytic activity also being increased [66,75]. AMACR overproduction appears to be mediated by a nonclassic C/EBP-binding motif in the promoter region [76]. Other enzymes involved in the peroxisomal β-oxidation of branched-chain fatty acids are also overproduced [e.g. acyl-CoA oxidase (ACOX)2, also known as *D*-bifunctional protein] [77], and that the relative levels of production of enzyme subtypes can also change (for example, ACOX3 expression is increased [77]), presumably due to increased levels of the substrates. Certain AMACR polymorphisms leading to single amino acid substitutions are also associated with increased prostate [78,79] and colon [80] cancer risk. In the case of prostate cancer, the strongest correlation is for the M9V polymorphism [79], with the minor allele overrepresented in unaffected men. Inactivating mutations in AMACR give rise to an adult-onset neurological syndrome [47,49,50], which is similar to adult Refsum's disease. As patients with these prostate cancer-related polymorphisms do not exhibit neurological symptoms, it implies that they do not abolish activity. Coupled with the overproduction of subsequent β-oxidation pathway enzymes, it implies that these cancer-related polymorphisms could misregulate the entry of metabolites into the pathway. Finally, there are several literature reports of overproduction of minor splice variants of AMACR in prostate cancer [81–83] (see below). These splice variants possess a common N-terminus but have different C-termini, and in some cases internal modifications towards the C-terminus. With the use of small interfering RNA techniques, reduction of AMACR production has been shown to prevent prostate cancer proliferation [66], suggesting that disturbances in branched-chain fatty acid metabolism are involved in the development or maintenance of the cancer. Although this study was performed before the existence of the minor splice variants was known, the small interfering RNAs were targeted to the C-terminal region, and would specifically reduce expression of AMACR 1A (the predominant form in 'normal' cells) and AMACR $1A_{DEL}$ [83], as the other variants do not contain the target sequence. The significance of the other splice variants in prostate cancer is therefore uncertain.

Biochemistry of AMACR

AMACR is colocalized in both peroxisomes and mitochondria in both humans [84,85] and rats [86]. The enzyme localized in both organelles is derived from a single transcript [84,86]. The enzyme possesses an N-terminal mitochondrial targeting signal and a C-terminal peroxisomal targeting sequence-1 variant, the final four amino acids, KASL [49]. These studies were performed before the existence of the minor splice variants [81–83] was known, and therefore refer to AMACR 1A, the major form of the enzyme in 'normal' cells. Examination of the minor splice variant sequences [81–83] reveals a common N-terminus containing the mitochondrial targeting signal. The C-terminal peroxisomal targeting sequence-1 signal is missing in all splice variants, implying that they will be exclusively mitochondrial, although this has yet to be verified.

The racemase-catalysed reaction requires no cofactors or cosubstrates [1,52,87,88], and involves stereospecific removal and addition of a proton. The formation of the CoA ester facilitates this process by increasing the basicity of the 2-proton (α-proton) by reducing the p*K*a from $\sim$ 34 to 21 [89]. Although this simple reaction could be theoretically performed without an enzyme, in practice the rates would be prohibitively slow and the alkali pH values would bring about hydrolysis of the CoA ester in preference to racemization. The reaction is reversible, and for the substrate containing a single chiral centre, the *in vitro* equilibrium constant has been measured as $\sim$ 1.5 (ibuprofenoyl-CoA with the rat enzyme) [52] in favour of the (2*R*)-isomer. As the fatty acyl components of the substrates/products are enantiomers, the chemical equilibrium constant might be expected to be close

to 1. This implies that a remote chiral centre in the CoA moiety favours formation of the *R*-isomer. Racemization is proposed to proceed via an enolate intermediate, and this is supported by studies using 2-$^{2}H_{1}$-labelled or 2-$^{3}H_{1}$-labelled substrates showing that label is lost during the reaction catalysed by the rat [53,87], human [88] and *Mycobacterium tuberculosis* [90,91] enzymes.

Although no X-ray crystal structure of a human or mammalian AMACR has been reported, amino acid sequence homologies show that AMACR is a member of the formyl-CoA:CoA transferase family (type III CoA transferases [92]), which includes *Escherichia coli* YfdW [93] and the CoA transferase from *Oxalobacter formigenes* [94]. These enzymes are dimers whose structures consist of two interlinked rings. Most recently, X-ray crystal structures of *M. tuberculosis* homologues of AMACR, MCR [90] and FAR [95], have been reported, which possessed the same overall fold. The structure of MCR was reported in conjunction with a site-directed mutagenesis study that identified some of the catalytic residues [90]. The study also looked at the effects of the equivalent mutations (I56P and M111P [90]) to those giving rise to AMACR deficiency in humans (S52P and L107P [49]). As expected, the M111P mutation led to a significant reduction in catalytic activity (to ~ 1.6% of wild-type activity). Unexpectedly, the I56P mutant had 76% activity as compared to the wild-type enzyme, when almost complete abolition of activity was expected. This anomalous result could reflect differences in the structures between the human and mycobacterial enzymes, or it may be that the S52P human mutant is significantly active and that racemase deficiency results from some other mechanism, e.g. reduced transcription or translation, or mRNA or protein instability.

The structural and mutagenic data enable some mechanistic details about the human AMACR-catalysed reaction to be predicted. However, the primary sequence identity of human AMACR 1A with these other enzymes is quite low, e.g. ~ 30% with MCR [90] and ~ 25% with YfdW [93], so any predictions should be treated with caution. It is noteworthy that the four important residues identified in MCR [90] are in regions of relatively high conservation. The equivalent residue to MCR Arg91 in AMACR 1A is Lys87; the MCR mutant displays an increased K_m value, suggesting that this residue is involved in CoA binding [90]. His126 in MCR is equivalent to His122 in AMACR 1A, and is highly conserved not just in racemases but also in other CoA-utilizing enzymes. His126 is the second base required for racemization, and probably stabilizes formation of the carbanionic intermediate. The residue is hydrogen-bonded to Glu241 from the second subunit, indicating that the active site is at the dimer interface [91]. It is noteworthy that the equivalent residue to MCR Glu241 is only found in racemase enzymes [90] (Glu237 in AMACR 1A). The second paper from the same group [91] reports the structures of MCR complexes with several acyl-CoA substrates. These structures support the previous proposals [90], and suggest a mechanism whereby Asp156 and the His126/Glu237 are involved in racemization (Fig. 1). The direction of catalysis appears to be controlled by the protonation states of the side-chains of these Asp and His residues [91]. There appears to be little structural change in the protein upon racemization, with the differences between the (2*R*)-substrate and (2*S*)-substrate arising due to swapping of the positions of the proton on the C^{α} atom and the C^{β} atom.

Exploitation of AMACR as an anticancer target is now possible, but surprisingly, only one paper has thus far appeared in this area [96]. The paper reported competitive inhibitors with K_i values of 0.9–20 μM when tested against enzyme purified from rat liver, with the

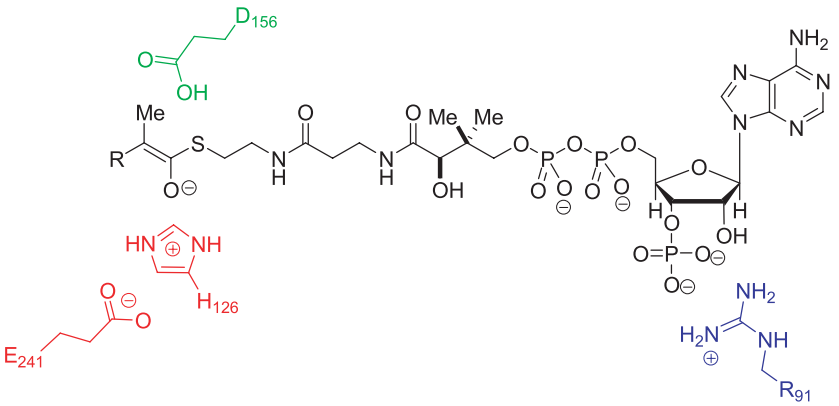


Fig. 1. Active site residues of human AMACR 1A identified from the *Mycobacterium tuberculosis* enzyme, MCR [90,91]. The catalytic residue is in green; the oxyanionic intermediate stabilization and proton acceptor residues are in red; the CoA-binding residue is in blue. The protonation state is for the (2*S*)-substrate to (2*R*)-substrate conversion.

most active compounds inhibiting growth of cancer cell lines. The potency of inhibition in cells is directly correlated with levels of AMACR protein in the cells. These results are encouraging, but a greater understanding of the roles of all the human splice variants is required in order for this approach to be fully exploited.

Unanswered questions and future work

Dietary branched-chain fatty acids represent a significant risk factor for prostate cancer, and the metabolic pathways responsible for degradation of these fatty acids are upregulated in cancers. AMACR acts as a 'gate-keeper' for β-oxidation. The identification of multiple splice variants implies a complex pathophysiological role for AMACR, and considering its recently discovered importance, relatively little biochemical work has been done. Major outstanding questions in this regard are whether these splice variants have catalytic activity and what their *in vivo* roles are in normal and/or cancer cells. The pathological link between dietary branched-chain fatty acids and cancer has not been determined, so it is not clear why branched-chain fatty acids appear to be more carcinogenic than straight-chain fatty acids. Peroxisomal β-oxidation is not linked to production of ATP in the same way that it is in mitochondria. The peroxisomal β-oxidation therefore results in the generation of reactive oxygen species, such as peroxide, and this probably explains the requirement for peroxidases, catalases, etc. in peroxisomes, from which the organelle gets its name. One theory on why branched-chain fatty acids are linked to cancer is that production of reactive oxygen species results in oxidative stress [97] leading to DNA damage. Support for this theory comes from a study showing that ibuprofen (a non-β-oxidizable substrate for AMACR) is protective against cataracts [98], which result from oxidative damage of lens proteins. Alternatively, it could be that branched-chain fatty acids or their metabolites are ligands for receptors involved in cancer. Phytol, phytanic acid and other branched-chain lipids are known to be high-affinity ligands for various receptors [99–104], including the PPARs [105–114] and retinoid X receptors [115–117], and are known to regulate expression of fat-metabolizing enzymes and brown fat tissue [118]. PPAR-α and PPAR-γ receptor agonists protect against cancer, whereas PPAR-δ agonists promote cancer in some animal models [119]. Phytanic acid [109] and pristanic acid [113] are agonists of PPAR-α, but their effects on PPAR-δ are unknown. Support for this model was recently provided by the observation that increased expression of FALDH-V protects cells against phytanic acid-induced damage in rodents [38]. This splice variant of FALDH performs the pristanal-to-pristanic acid conversion in the α-oxidation pathway, thus facilitating detoxification of phytanic acid and its phytol precursor. However, this area is complicated by the considerable differences between rodent and human PPAR pathways as well as between tissues. For example, phytol [111,114] may be a PPAR-α ligand in human cell lines, whereas phytanic acid is a PPAR-α ligand in mice [103] but its effects in humans are controversial. It could be that branched-chain fatty acids or their metabolites are agonists for PPAR-δ or antagonists for PPAR-γ, and this is the molecular basis for cancer formation, at least in some model systems. These theories merit further investigation and are attractive in the sense that they explain why particular cancers appear to be promoted, as prostate and breast tissues are particularly active in fat metabolism.

Selective inhibition of specific splice variants could lead to new anticancer therapies. The use of AMACR inhibitors is particularly attractive, as protein expression levels can be measured and appear to correlate with disease progression. The fact that the target of these inhibitors is used as a marker raises the possibility of molecular targeted therapies, especially in those cancers where AMACR is overproduced in a subpopulation of patients (e.g. gastric adenocarcinomas [61]). AMACR-knockout mice appear to healthy in the absence of branched-chain fatty acids in the diet, but develop symptoms in their presence (phytol) [51]. Some adult Refsum's disease symptoms can be reduced in human patients on a low-phytanic acid diet [2], suggesting that the undesirable side-effects of AMACR inhibition could be minimized by dietary therapy. However, in order for AMACR to be developed as a successful anticancer drug target, the catalytic activities of the various splice variants need to be determined. If AMACR inhibitor therapy is to be used more generally in anticancer therapy, the expression of the various splice variants in other cancers will need to be determined. In the shorter term, the identification of AMACR polymorphisms increasing prostate cancer risk [78,79] could provide screening opportunities.

Prostate cancer is an important and complex disease of Western society, with 218 890 men in the USA being diagnosed in 2007, with 27 050 deaths (9% of all male cancer deaths) [120], and 31 900 men in the UK being diagnosed (23% of all male cancers) in 2003 (Cancer Research UK: http://www.cancerhelp.org.uk/help/default.asp?page=2656). Preliminary epidemiological studies have shown that lower phytanic acid

intakes are associated with lower rates of prostate cancer [73,121]. Diets with low phytanic acid have been available for many years for the treatment of adult Refsum's disease [122–124]. A recent study was performed as part of an EU project on adult Refsum's disease, and the website contains a phytanic acid calculator for various foodstuffs in resources for both patients and clinicians (http://www.refsumdisease.org). A reduced phytanic acid diet could be of benefit to men at risk of developing prostate cancer and be of use for prevention of other major cancers, such as those of breast and colon. Plasma phytanic acid levels are strongly associated with dairy fat intake [32], with the levels found in meat eaters, lacto-ovo-vegetarians and vegans being 5.77, 3.93 and 0.87 μM, respectively. Restriction of intake of dairy fats, animal fats and fish oils is a simple and effective method of reducing phytanic acid intake.

In the wider context, branched-chain fatty acid metabolism could have wide-reaching implications. The number of structures that could be theoretically metabolized by this route is large (in some cases, preliminary metabolism by ω-oxidation is required). These include fat-soluble vitamins such as vitamin E and many plant sterols and fats. This implies that a large number of dietary fats could be either protective or procarcinogenic.

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