Isoprenoid biosynthesis and mevalonate kinase deficiency

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General discussion and summary
The isoprenoid biosynthesis pathway is an important metabolic pathway which produces a range of sterol and nonsterol isoprenoids, vital for multiple cellular functions. Isoprenoids such as ubiquinone-10, heme A, dolichol, isopentenyl tRNA, the farnesyl and geranylgeranyl groups of isoprenylated proteins and cholesterol are incorporated into diverse classes of end products that participate in processes relating to cell growth, differentiation, glycosylation, isoprenylation and various signal transduction pathways. Several inherited disorders have been linked to specific enzyme defects in isoprenoid biosynthesis, however, only one disorder affects the biosynthesis of all isoprenoids since it occurs early in the pathway. This disorder is mevalonate kinase deficiency (MKD). MKD is an autosomal recessive metabolic and autoinflammatory disorder characterized by life-long episodes of high fever and inflammation. MKD is caused by mutations in the MVK gene resulting in decreased activities of mevalonate kinase. Dependent on the mutations, patients may present with the hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) or classic mevalonic aciduria (MA), which represent the mild and severe clinical and biochemical ends of the MKD spectrum. HIDS is characterized by recurrent episodes of fever, which usually return every 3 to 6 weeks and last for 3 to 7 days. These fever episodes are associated with malaise, headache, arthralgias, arthritis, nausea, abdominal pain, diarrhea, skin rash, hepatosplenomegaly and lymphadenopathy. Patients with the severe MA presentation experience similar fever episodes; however, in addition they have congenital and developmental features, and may die in early childhood. The attacks can be triggered by vaccinations, infections, minor trauma and physical or emotional stress, but most often such a trigger can not be identified. Chapter 1 is a review of the current knowledge on the isoprenoid biosynthesis pathway, its defects with emphasis on MKD, the isoprenylation of small GTPases, and \textit{in vivo} models of MKD.

MKD patients with the mild HIDS presentation can be divided in two subgroups: patients with “variant type HIDS”, which have clinical symptoms indicative of HIDS, but no mutations in the MVK gene, and patients with “classic type HIDS”, which do have mutations in the MVK gene. Subtle differences in symptoms, signs and laboratory findings were noted upon comparison of HIDS variants with classic type HIDS patients. However, the defect or defects in the variant type patients which result in periodic fever are unknown at present. One of the possibilities is that in addition to MK, defects of other enzymes of the isoprenoid biosynthesis pathway could also cause periodic fever. Therefore, we developed a sensitive method using HPLC-MS/MS (chapter 2) and UPLC-MS/MS (chapter 3) that allows the direct detection and quantification of all intermediates of the mevalonate pathway. Chapter 2 describes the validation of the method and also demonstrates that by blocking the isoprenoid biosynthesis pathway with pamidronate, an inhibitor of farnesyl pyrophosphate synthase, accumulation of intracellular levels of pathway intermediates can be determined in a time-dependent manner in HepG2 cells. Analysis of (cultured) cells, PBMCs or tissue by our HPLC-MS/MS method therefore may be helpful to identify the defect in variant type HIDS patients. In addition, our method can also be a useful tool in determining the specificity of inhibitors of the isoprenoid biosynthesis pathway. Since the isoprenoid biosynthesis pathway is an important target in many areas of ongoing research, new inhibitors to block this pathway are being developed. Inhibition of this pathway is already applied
in the treatment of cardiovascular disease, hypercholesterolemia and metabolic bone
disease and is a possible new therapy in cancer treatment. In chapter 3 we tested
several inhibitors of the isoprenoid biosynthesis pathway. We demonstrated that some
inhibitors specifically inhibit one enzyme of the isoprenoid biosynthesis pathway, like
the nitrogen containing bisphosphonates pamidronate and zoledronate, while other
inhibitors, such as zaragozic acid A and 6-fluoromevalonate, have an effect on multiple
enzymes of the pathway, either direct or indirect through accumulation of isoprenoid
intermediates. These results show that our UPLC-MS/MS method can be a useful tool in
determining the specificity of inhibitors of the isoprenoid biosynthesis pathway.
Mevalonate is the pathway intermediate which accumulates in MKD and, indeed,
patients have elevated levels of mevalonate in plasma and urine. However, there is
also a shortage in isoprenoid end products when MK is deficient and, in particular,
the synthesis of geranylgeranyl pyrophosphate appears to be compromised in
MKD. Because small GTPases are highly dependent on geranylgeranylation (i.e.
isoprenylation) for their proper signaling function and have been implicated in the
regulation of inflammatory processes, we studied the effect of MK deficiency on the
geranylgeranylation, activation and localization of the three small Rho-GTPases RhoA,
Rac1 and Cdc42. Protein isoprenylation is usually rather normal when cells of MKD
patients are cultured under normal conditions, even though the MK enzyme activity
can be hardly detectable in these cells. This is due to an increased activity of HMG-
CoA reductase, the rate-limiting enzyme of the isoprenoid biosynthesis pathway, which
leads to elevated levels of mevalonate and a virtually normal flux through the pathway.
However, this pathway flux in MKD cells is very sensitive to small disturbances. Because
MKD cells depend on elevated levels of mevalonate to maintain the flux through the
pathway, they are more sensitive to simvastatin, an inhibitor of HMG-CoA reductase.
Chapter 4 describes that both geranylgeranylation and activation of RhoA and Rac1 is
indeed more easily disturbed in MKD cells than in control cells when the flux though the
isoprenoid biosynthesis pathway is suppressed by low concentrations of simvastatin.
The limited capacity of geranylgeranylation in MKD cells readily leads to markedly
increased levels of nonisoprenylated, and activated GTPases, which will affect proper
signaling by these GTPases. In chapter 5 we studied the effect of elevated temperatures
on the localization and activation of RhoA, Rac1 and Cdc42 in MKD. Not only because
high fever is the most prominent symptom in MKD, but also because we previously
found that a small increase in temperature already results in a rapid further decrease in
the residual MK enzyme activity in MKD cells and consequently in an instant block in the
isoprenoid biosynthesis pathway leading to a shortage of end products. We observed
that incubating fibroblasts at 40°C (i.e. mimicking a fever episode) induces an altered
subcellular distribution of RhoA, Rac1 and Cdc42 in cells from MKD patients but not
in control cells. In addition, the elevated temperature results in a markedly increased
activation of these soluble GTPases. We postulate that such ectopic activation of small
GTPases gives rise to inappropriate signaling, which may underlie the inflammatory
presentation observed in MKD.
Chapter 6 describes the generation and characterization of two mouse models that
present close genocopies of human MKD. The generated mice are either homozygous
for the V377I mutation in the Mvk gene, which is the most commonly observed mutation
associated with HIDS in humans, or compound heterozygous for the V377I mutation
and a deletion of exon 10 and 11 in the Mvk gene. Biochemical characterization of
the two mouse models indicate that they represent good phenocopies for the human disorder and thus will be suitable for studies to the pathophysiology associated with MKD.

Since there is currently no general efficacious treatment available for MKD, the mouse models described in this thesis will be useful to test and develop therapeutic interventions. We showed that the small GTPases RhoA, Rac1 and Cdc42 have an altered subcellular distribution and disturbed activation in MKD cells, which probably is associated with the onset of fever episodes and inflammation in MKD patients. Because this is the result of a temporary shortage of geranylgeranyl pyrophosphate, manipulation of the isoprenoid biosynthesis pathway would be a potential therapeutic approach for the treatment of MKD. A possible therapy could be supplementation of intermediate isoprenoids (e.g. farnesyl pyrophosphate and geranylgeranyl pyrophosphate), specific enzyme inhibitors, including squalene synthase inhibitors (e.g. Zaragozic acid A) that redirect the flux through the pathway to nonsterol isoprenoid biosynthesis, and ligands that are known to upregulate the isoprenoid biosynthesis pathway, such as sterol-regulatory element binding protein (SREBP)-activating SCAP ligands.

The question remains how a disturbance in isoprenoid biosynthesis leads to periodic fever and inflammation. In this thesis we demonstrated that stress, including elevated temperature, results in an altered subcellular distribution of activated small GTPases in MKD cells. This may lead to inappropriate signaling, i.e. failure to induce certain signaling pathways or incorrect induction of other signaling pathways involved in the regulation of the innate immune response or both. Which isoprenylated GTPase(s) and which signaling pathways are affected in MKD, however, is still unknown. Although we consider it likely that either of the GTPases RhoA, Rac1 or Cdc42 plays a role in MKD, we do not exclude that other less well studied GTPases are involved. Transcriptome- and phosphoproteome profiling in MKD cells may point out which signaling pathways are altered in MKD. Moreover, the results of such studies should indicate signaling pathways that are connected with the function of GTPases that could play a role in the pathogenesis of MKD.

Another important question is whether the NALP3 inflammasome1 is involved in the onset of periodic fever and inflammation in MKD. MKD has been assigned to the group of autoinflammatory diseases and, in the majority of these disorders, pathogenic mutations have been identified in genes encoding components of the NALP3 inflammasome or factors known to modulate the assembly or activation of the NALP3 inflammasome. These inflammasomes are multi-protein complexes that assemble upon sensing danger and serve as molecular scaffolds for the dimerization and subsequent activation of procaspase-1. Caspase-1 in turn is required for the proteolytic processing and subsequent release of active pro-inflammatory cytokines, such as IL-1β, IL-18 and IL-33 and thus is a critical component in the regulation of innate immunity. Like in other autoinflammatory diseases, IL-1β appears to play an important role also in the pathogenesis of MKD, as indicated by the massive ex vivo production of this pyrogenic cytokine observed after LPS stimulation of PBMCs from MKD patients. This increased IL-1β production is reversed by supplementation of downstream isoprenoid intermediates, including geranylgeranyl pyrophosphate. The increased IL-1β secretion observed in PBMCs from MKD patients suggests that MK deficiency may give rise to a rapid activation of NALP3 inflammasomes, which results in activation of caspase-1 required for proteolytic conversion of pro-IL-1β activation. To determine if there is a
connection between MK deficiency and NALP3 inflammasome function or activation, our MKD mouse model described in chapter 6 will be very useful. After crossing our MK-deficient mice with functional inflammasome-depleted NALP3 knock out mice, it would be interesting to analyze peritoneal macrophages for their capability to secrete IL-1\(\beta\) following LPS stimulation. A disability to secrete IL-1\(\beta\) upon stimulation would point to a connection between the compromised isoprenoid biosynthesis in MKD and functioning of the NALP3 inflammasome. However, when IL-1\(\beta\) secretion is still observed on the inflammasome-depleted background, this would point to a different mechanism.

The exact mechanism by which MK deficiency leads to the recurrent episodes of fever and inflammation and whether there is a direct link with NALP3 inflammasome is still unclear. Therefore, future research should focus on this important issue and hopefully will lead to the finding of novel targets for the treatment of MKD.