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AMPK signaling in dendritic cells: a metabolic sensor controlling the balance between immunity and tolerance

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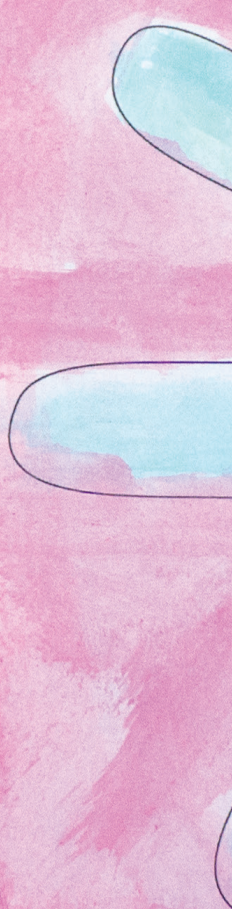
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Part 1

Introduction





1

General introduction

Introduction

All living organisms need energy for survival, development, and growth. Nutrients are a major energy source and therefore sufficient nutrient availability is indispensable for life. The capacity to adapt intracellular processes to extracellular nutrient levels is crucial to ensure optimal growth and survival. Even prokaryotes have receptors and enzymes that respond to fluctuating extra- and intracellular nutrient levels. The engulfment of oxidative bacteria and the capacity to store nutrients in subcellular compartments required more complex metabolic regulation in eukaryotes (1). One of the pathways involved in metabolic control that is absent in prokaryotes, but conserved among all eukaryotes is the AMP-activated protein kinase (AMPK) signaling axis (2). AMPK is activated upon a variety of metabolic stimuli and acts through phosphorylation of key metabolic enzymes and regulators of cellular growth, thereby adapting intracellular metabolism and processes to energy levels and nutrient availability. AMPK consists of a catalytic α -subunit, and regulatory β - and γ -subunits. This heterotrimeric structure is conserved among eukaryotes, but upstream and downstream regulators can differ. For example, canonical AMPK activation in mammals is facilitated by binding of adenine nucleotides AMP and ADP to the γ -subunit, while only ADP can activate *S. cerevisiae* AMPK homolog Snfl (sucrose nonfermenting). However, similar to mammalian AMPK, Snfl also promotes a switch to oxidative metabolism (3,4). Plant homolog SnRK1 (SNF1-related kinase) is not activated by adenine nucleotides, but inhibition by sugar-phosphates (e.g. glucose-6-phosphate) plays a major role in regulating its activity. Interestingly, SnRK1 is also activated in the dark, which could be considered an energy-deprived condition for plants (5). This indicates that the AMPK signaling pathway evolved to tailor the needs of an organism, also evidenced by a role for AMPK in the hypothalamus of animals, where it controls whole-body homeostasis by promoting food intake and body weight (6). Despite the regulatory differences between species, the main purpose of AMPK signaling is conserved from unicellular eukaryotes to more evolved species: maintaining energy homeostasis during metabolic stress.

Canonical AMPK activation, mediated by adenine nucleotide binding, involves three mechanisms: allosteric activation, promotion of phosphorylation of Thr183 (α 1-subunit) or Thr172 (α 2-subunit) by liver kinase B1 (LKB1) (7), and inhibition of Thr183/Thr172 dephosphorylation (4,8). AMPK can additionally be phosphorylated by other kinases, including Ca²⁺/calmodulin-dependent kinase kinase β (CaMKK β) upon Ca²⁺ accumulation (9) and transforming growth factor- β -activated kinase 1 (TAK1) upon lysosomal damage (10) (Fig. 1). Non-canonical modulation of AMPK activity mediated by fluctuating nutrient levels. High glucose levels (11) and potentially glycogen levels can inhibit AMPK (8), while long-chain fatty acid (LCFA)-CoA esters (the activated, soluble form of LCFAs) (12) and microbiota derived short-chain fatty acids (SCFA) (13) can function as AMPK activator.

Over a 100 AMPK target proteins have been reported, that impact a wide variety of metabolic pathways and cellular functions. Promoting glucose uptake, induction of fatty acid oxidation, and boosting mitochondrial biogenesis are among the best-studied downstream effects of AMPK signaling, as well as autophagy and inhibition of protein synthesis (Fig. 1) (8,14). Altogether, these processes aim to maintain sufficient cellular energy levels during metabolic stress.

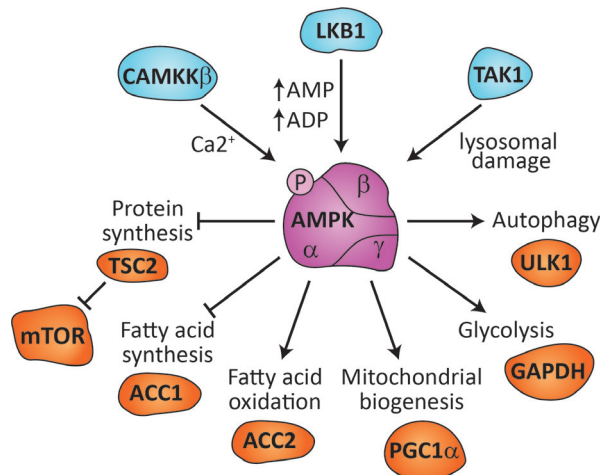


Figure 1: AMPK signaling pathway. Ca²⁺/calmodulin-dependent kinase kinase β (CaMKKβ), Liver kinase B1 (LKB1), and transforming growth factor-β-activated kinase 1 (TAK1) activate AMPK in response to rising Ca²⁺ levels, increases in ADP or AMP levels, and upon lysosomal damage respectively. Key targets of AMPK include Tuberous Sclerosis 2 (TSC2), Acetyl-CoA Carboxylase (ACC) 1 and ACC2, PPARγ Coactivator 1 Alpha (PGC1α), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and Unc-51 like autophagy activating kinase (ULK1).

During the past decade it has become clear that cellular metabolism directly affects the function of immune cells, including that of dendritic cells (DCs) (15,16). DCs are antigen presenting cells that upon activation can promote inflammatory as well as anti-inflammatory immune responses by governing the priming and polarization of T cell responses (17). A number of DC subsets, including conventional DCs, plasmacytoid DCs, monocyte-derived DCs, and skin-resident Langerhans cells are recognized, that differ in their tissue distribution, function, and ontogeny (18). These DC subset-defining characteristics, as well their activation status, are associated with distinct metabolic profiles (15,16). Generally speaking, induction of pro-inflammatory T cell responses are associated with anabolic metabolism in DCs, including upregulating glycolysis and fatty acid synthesis (19,20), while DCs conditioned to prime T cells towards a regulatory phenotype (21,22), as well as DCs in the immunosuppressive tumor-micro environment (23), display a catabolism-centered metabolism, characterized by increased glycolysis, oxidative phosphorylation, and fatty acid oxidation (FAO) (Fig. 2).

Several studies point towards a key role for AMPK signaling in DC-driven immune responses. For instance, AMPK activation in DCs dampens LPS-mediated activation (19) and loss of AMPK in DCs has been shown to promote LPS-induced activation (24). Furthermore, AMPK signaling in CD11c⁺ myeloid cells contributes to Th2-driven control of hookworm infection (25). Yet, although the metabolic phenotype observed in tolerogenic DCs is similar to AMPK-induced metabolic changes (14), it remains unknown whether AMPK signaling in DCs shapes their ability to orchestration of regulatory immune responses and what the underlying (metabolic) mechanisms are through which AMPK shapes DC-driven T cell responses.

Within the framework of this thesis, we aim to address the hypothesis that DC-intrinsic AMPK signaling governs the balance between inflammatory and tolerogenic immune responses by controlling cellular metabolism. In addition, we question whether AMPK in DCs serves a

key nutrient sensing role that functionally connects metabolic conditions of the local micro-environment to changes in T cell priming properties of DCs (Fig. 2). Our goal is to contribute to provide a better understanding of how this metabolic sensor in DCs controls the balance between immunity and tolerance and how ultimately targeting AMPK in DCs may contribute to improved treatment of inflammatory and metabolic diseases.

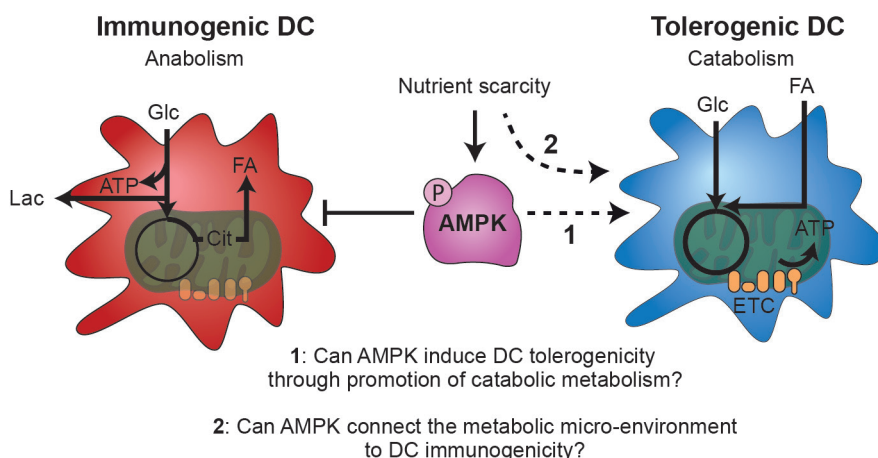


Figure 2: AMPK signaling in dendritic cells: research questions. Metabolic pathways associated with immunogenic and tolerogenic DCs, and research questions addressed in this thesis, regarding the role of AMPK in driving DC metabolism and immunity.

Thesis outline

Chapter 2 and chapter 3 constitute a theoretical and practical introduction to this thesis. In **chapter 2** a background is provided on DC subsets, function, and metabolism, and the current knowledge about the effects of the metabolic micro-environment on DC biology is described. In **chapter 3** a protocol to study intracellular metabolism in DCs using flow cytometry is given. The described methods are used in all research chapters of this thesis and are essential tools to perform research in the field of immunometabolism.

In **chapter 4** the role of the AMPK signaling axis in an inflammatory context is addressed. Obesity causes chronic low-grade inflammation in metabolic organs (26) and here we showed an important role for the LKB1-AMPK/SIK signaling axis in hepatic DCs, in controlling metabolic homeostasis during obesity.

The next section focusses on the role of AMPK in DCs as regulator of tolerance. In **chapter 5** we described how drug-induced AMPK activation induces metabolic changes that lead to RALDH^{high} tolerogenic DCs. In **chapter 6** we examined the tumor micro-environment as physiological activator of AMPK and showed that AMPK activation in tumor-associated DCs compromises the control of tumor growth. In **chapter 7** we described how the effects of

tolerogenic compound retinoic-acid depends on AMPK signaling and how AMPK activity in DCs *in vivo* induces regulatory T cell responses.

In **chapter 8** the main findings of this thesis are summarized, novel insights are discussed in a broader perspective, and future research suggestions are provided to expand our knowledge on the role of AMPK signaling in dendritic cells.

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