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mTOR: dissecting regulation and mechanism of action to understand human disease

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Abstract

This timely meeting brought together groups with shared interests in the regulation and effects of the mammalian Target Of Rapamycin (mTOR, see Figure 1), a highly conserved serine/threonine protein kinase with roles in cell metabolism, cell growth and cell survival. Although for some years it has been known that mTOR acts as a hub for inputs from growth factors (in particular insulin and insulin-like growth factors), nutrients and cellular stresses, some of the mechanisms involved are still poorly understood. Recent work implicating mTOR in a variety of important human pathologies, including cancer, Type 2 diabetes and neurodegenerative disorders has heightened interest and accelerated progress in dissecting out the control and functions of mTOR. An important goal of this meeting was to bring together individuals approaching the key problems from very different perspectives. In particular discussion of work addressing the role of nutrient sensors and transporters in mTOR regulation, brought a novel flavour to this TOR-centric meeting, which was welcomed by many delegates. The meeting was divided into three sessions, all intimately linked: 'Insulin/TOR signalling and translational control', 'Nutrient regulation of mTOR' and 'The mTOR signalling pathway: physiology, pathology and treatments'. The scientific programme resulted in a lively and interactive meeting with extensive discussion following talks and also around posters. It was especially useful in raising some of the remaining questions that have yet to be fully addressed and in highlighting recent work that may ultimately help to provide several of the answers. Since the discovery of TOR proteins in yeast by Mike Hall and colleagues in the early nineteen-nineties working in Basel, more than 3000 papers on mTOR have been published; moreover, as Michael Hall indicated in his excellent introductory lecture, the literature is still expanding exponentially. It is only the newer areas covered at this meeting that are very briefly reviewed here.

Cell type-specific functions of the mTOR signalling cascade

Since mTOR plays two distinct functions in metabolism and growth factor signalling through TORC1 and TORC2 and has a critical role in endocrine functions in multicellular organisms, the 'simple' knockout studies in mice that have primarily been performed to date have not fully untangled the functional complexity of this molecule. Mike Hall described the first characterisation of animals with conditional tissue-specific knockouts of the TORC1 component *raptor* and the TORC2 component *ricor*, which highlighted complex endocrine interactions between different tissues [1]. There is little doubt that these types of study will complement work, such as that described by Brendan Manning [2], Joe Avruch [3] and Chris Proud [4] where the roles of insulin and mTOR signalling components are dissected in cell lines or cultured cells derived from mutant mice

The cell biology of amino acid-dependent TOR activation

Recent work from the Sabatini laboratory presented by Yasemin Sancak has revealed that in HEK-293T cells, mTOR is shuttled to Rab7-containing late endosomes in the presence of

activating extracellular amino acids, suggesting that this might be part of the activation mechanism [5]. These late endosomes contain the upstream growth factor-regulated G protein Rheb, which synergises with amino acids in cultured cells to maximally activate mTOR. Not only has this study indicated that the endosomal system is important at least in amino acid-dependent TOR activation, but it has also emphasised how important it will be in the future to consider the subcellular control of TOR and its activities. It will be interesting to see whether AMPK discussed by Grahame Hardie [6, 7] and Ruben Shaw [8], or the hypoxic conditions described by Celeste Simon [9] are involved in regulating the same or a different pool of mTOR.

Amino acid sensing and mTOR regulation

Several recent studies in cell culture have put forward different molecules as sensors for amino acids involved in mTOR activation. These include MAP4K3 [10], Vps34, a lipid kinase controlling phosphoinositide metabolism [11, 12] and Rags [5]. Speakers at the meeting put forward several other candidates. For example, *Egln* prolyl hydroxylases are inactivated by reduced levels of α -ketoglutarate that are associated with amino acid starvation, and Raul Duran suggested that this inactivation is important in loss of mTOR activity [13]. Activity of the p38 stress-activated MAP kinase pathway was also shown by Megan Cully to play a role in activating mTOR in flies and cell culture [14]. And our own labs presented work supporting a role for the proton-assisted amino acid transporters (PATs) as TOR-activating amino acid sensors [15]. Since these transporters can function in endosomal compartments, this study correlated well with the findings of Yasemin Sancak, who showed that Rag GTPases are required for amino acid-dependent mTOR activation and shuttling of mTOR to endosomes [5]. It will be interesting to determine whether the Rags and PATs work via a common mechanism.

Another interesting aspect of the meeting related to the discussion of mechanism by which molecules that look like amino acid transporters might signal intracellularly. Both work from the Goberdhan and Boyd labs on the PATs [15] and from Peter Taylor's lab on another amino acid transporter family, the SNATs [16], has indicated that some of these molecules may not need to transport to activate their targets, acting as so-called transceptors. Clearly establishing the signalling mechanism involved is a priority, and Per Ljungdahl gave a comprehensive demonstration of how this had been achieved in yeast for the SPS amino acid sensor [17]. Furthermore, Manuel Palacin described a structure-function analysis of the heterodimeric amino acid transporters [18], illustrating how a detailed structural analysis of transporters as well as a determination of the substrate binding and specificity of novel classes of transceptor are likely to inform our understanding of mechanism and perhaps allow design of new inhibitory drugs. It will also be important to find out how the levels of amino acid transporters are regulated and whether these levels can be modulated. Aimee Edinger presented evidence suggesting that ceramide, which has been shown to down-regulate nutrient permeases in yeast, also induces autophagy with potential therapeutic applications for targeting cancer cells [19]. The significance of tissue-specific amino acid transporters to normal human development was also aired by Sara Roos, for example in the placenta [20].

What pathways work downstream of TORC1?

Classically TORC1 links to pathways and molecules controlling mRNA translation, such as S6-kinase and the 4E-BP. However, TORC1's inhibitory effects on autophagy also affect the response of cells to amino acids. Tom Neufeld presented recent data dissecting out the roles of different *Atg* genes in the autophagy process that indicated more complexity in the fusion and protein shuttling events involved than previously had been suspected [21].

Furthermore, Almut Schulze described a role for TORC1-dependent SREBP accumulation in the nucleus in mediating the growth-promoting effects of mTOR and growth factor signalling [22]. SREBP is necessary for increased lipid biosynthesis required for cell growth and thus this work implies that this neglected aspect of mTOR function will be as important to study as the protein translational mechanisms that have been extensively analysed in the past. In addition, Janni Petersen presented data from fission yeast linking TOR signalling to mitotic commitment in response to nutrient availability [23].

Is drugging the TOR pathway useful therapeutically?

Julian Sampson and Michel Maira presented pre-clinical and/or early-phase human trials indicating that inhibiting mTOR might be effective in treating a range of TSC-associated tumours, and that there were likely to be advantages in inhibiting both PI3K and TOR in some cancer therapies [24, 25]. It was not only gratifying to hear that work that has developed from combined studies in model organisms as diverse as yeast, flies, mouse and human is now influencing treatment strategies, but importantly these studies highlighted the point that further intensive study of the mTOR control and downstream signalling mechanism, such as that described in the rest of the meeting, was likely to drive further progress in the clinic in the not-too-distant future. The reviews that follow will hopefully illustrate where future breakthroughs may emerge.

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Figure 1. mTOR signalling, nutrients and disease

The mammalian Target Of Rapamycin, mTOR, regulates protein translation via effects on S6K and 4E-BP to modulate cell and organismal growth. It also suppresses autophagy, the process by which cells can turnover organelles under stress-activated conditions. Autophagy has been implicated in the breakdown of aggregates that can accumulate in some neurodegenerative disorders²¹. mTOR is known to exist in two complexes, mTORC1 with Raptor, and mTORC2 with Rictor. mTORC1 is regulated by the highly conserved growth factor signalling cascade involving phosphatidylinositol 3-kinase (PI3K), the serine/threonine kinase Akt (also known as PKB) and the G-protein, Rheb. PI3K/Akt signalling, which is antagonised by the major human tumour suppressor gene, PTEN, is known to be upregulated in the majority of human cancers and also to affect processes involved in diabetes. The drug rapamycin, which has well established effects on mTORC1 signalling, now also appears to affect mTORC2 signalling². Modulating the regulation of mTORC1 by local inputs such as glucose levels, via the energy sensor AMPK, or amino acids, presumably via amino acid transporters (AATs), or oxygen levels, has the potential to have important therapeutic implications.