Review

UHN Microarray Centre

Identification and characterisation of a novel gene, DAPR

Review of: Virtanen C, Paris J, Takahashi M. Identification and characterization of a novel gene, DAPR, involved in skeletal muscle differentiation and PKB signalling. J Biol Chem 2008, November 20 [Epub ahead of print]

Phosphatidylinositol 3-kinase (PI_3K) signalling is one of the key pathways involved in the transformation of myoblasts to myotubes (1). Although the PI_3K signalling pathway is well characterised, little is known about how its activation coordinates specific cellular events (2). Protein kinase B (PKB) is one of the downstream effectors of PI_3K signalling, and besides its role as signal transducer, it also plays an important role in cellular differentiation (1,3,4). The purpose of this study was to understand how a protein like PKB, which is also a key protein in many other signalling pathways, can be directed to a specific biological event like differentiation. This investigation led Virtanen and colleagues to identify a novel gene, Differentiation Associated Protein (DAPR; also known as PLEKHM3), which they suggest is involved in the PI_3K pathway by potentially acting as a scaffold protein for PKB and coordinating its compartmentalisation during skeletal muscle differentiation (2).

In a previous study using chromatin immunoprecipitation followed by CpG microarray analysis (ChIP-chip), DAPR was found to be regulated by the muscle related transcription factor MEF2 during skeletal muscle differentiation (5). This study serves to characterise DAPR and identify its potential role in muscle differentiation.

DAPR was characterised *in silico* using a number of tools to find conserved domain structures. PFAM (database of Protein FAMilies; 6) matrices predicted two PH (pleckstrin homology) domains and the SMART (Simple Modular Architecture Research Tool; 7) database search also predicted a C1 (protein kinase C conserved region 1) binding domain located near the carboxy-terminal end of DAPR. PH domains are reportedly involved with phosphoinositide binding and membrane targeting (8). Since PKB has a PH domain, binds phosphoinositides (9), and plays a role in muscle differentiation via the PI_3K signalling, Virtanen and colleagues hypothesised that DAPR could be associated with PI_3K signalling, specifically by binding to PKB.

The experimental data from this study shows that DAPR is localised mostly in the Golgi apparatus in undifferentiated myoblasts but moves to the membrane during differentiation. The addition of a membrane targeting myristoylation tag to PKB resulted in DAPR also moving to the membrane and another experiment showed that PKB co-precipitated with DAPR. Further evidence that DAPR is involved in myoblast differentiation comes from DAPR knockdown experiments. Following RNA interference by transfection with siRNA oligos, myoblast cells had a significant reduction of DAPR protein at the time of differentiation. This study also shows that PKB was absent from the membranes of siRNA transfected cells. On a global level, knockdown of DAPR had a significant effect on the phenotype of cultures of differentiating myoblasts, with fewer formations of myotubes present. These results led Virtanen and colleagues to propose that DAPR acts as a scaffold protein for PKB.

Interestingly, homologous genes or gene fragments for DAPR are found across all sequenced Vertebrata, although none have been characterised. DAPR also has 49% homology to a related protein, PLEKHM1; a component of osteoclast formation that is involved in osteopetrosis (10).

The data presented in this study serves as an initial characterisation of DAPR. The implication of DAPR being involved with such an extensive signalling pathway as the PI_3K signalling cascade suggests that it is a worthy candidate for further studies.

References

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