Seminar

Institute for Plasma Research

Title : CNK1 scaffold protein and its role in the regulation of NF-κB signaling

Speaker : Dr. Kshama J Pansare
Tata Memorial Centre, Mumbai

Date : 17th November 2017 (Friday)

Time : 10.30 AM

Venue : Seminar Hall, IPR

Abstract :

Discovering the underlying mechanisms of signalling pathways has always been a challenge to researchers. The harmonious environment in which they co-ordinate their activity makes it even more difficult to interpret the final effects. We have studied the role of Connector Enhancer of KSR (CNK1) scaffold protein, in modifying the activity levels of transcription factors (TFs) involved in MAPK and NF-κB oncogenic signalling pathways. Scaffold proteins increase the throughput in signal transduction pathways resulting in more efficient cellular signalling. When scaffold proteins alter the levels of TF activity in the cell, they result in the modification of the cellular transcriptome profile. Significant information can be derived from the study of these profiles. A library of sensor plasmids encoding transcription factor binding sites (TFBS) upstream of unique reporter sequences (UR) was transfected into HeLa cells in presence and absence of the CNK1 scaffold protein. TF activity was quantified using qPCR and validated at protein level with Western blots. An increase in the cellular CNK1 concentration resulted in changes in the activity of some TFs (i.e. AP-1 and CREB) but not others (i.e. NF-κB). The upand down-regulation of TFs provided an insight into the complexity of cellular pathways and the diverse roles for a scaffold protein in the cell. The validity of the TF activity data was confirmed by using NF-κB inhibitors to knockdown NF-κB activity. A remarkable discovery was made while doing so; sulfasalazine, a specific NF-κB inhibitor targeted the CNK1 scaffold protein for proteasomal degradation. The addition of a proteasomal inhibitor, MG132, resulted in the recovery of the CNK1 scaffold protein levels in HeLa cells. The role of CNK1 in the canonical and non-canonical NF-κB signalling pathways was investigated further and CNK1 was shown to partially down-regulate both pathways. We have demonstrated that the scaffold protein CNK1 has a concentration-dependent effect on TF activity in the cell; that sulfasalazine is a potential CNK1 scaffold protein inhibitor and that CNK1 is involved in regulation of the NF-κB signalling pathways.