## Summary

CD40 is an important co-stimulatory molecule expressed by DCs, Mφ and microglial cells. Its aberrant expression has been implicated in various diseases including multiple sclerosis, rheumatoid arthritis, and Alzheimer's disease. In addition, CD40 plays a dominant role in regulating activation/maturation of DCs and influences the outcome of T cell response. Because CD40 has multifunctional roles in CMI and humoral immune responses, it is likely that expression of CD40 is strictly regulated at various levels in DCs. However, the molecular mechanisms that regulate *Cd40* transcription in DCs are largely unknown.

Previous studies have shown that expression of other co-stimulatory molecules such as CD86 and CD49d is regulated by the runt-related (RUNX) family of transcription factors in DCs. Additionally, recent studies have demonstrated that RUNX proteins play key roles in regulation of immune response. The RUNX family consists of three members RUNX1, RUNX2 and RUNX3. The roles for RUNX factors in DC immunobiology have been described by limited number of studies. However, the roles for RUNX factors in transcriptional regulation of *Cd40* in DCs remain unrecognized. Accordingly, the current study was initiated to analyze the involvement of RUNX proteins, if any, in regulation of CD40 expression in murine dendritic cells. Furthermore, whether RUNX proteins, by influencing CD40 expression, contribute to DC activation was investigated.

Most of the experiments in the present study have been done on BMDCs or sDCs of mouse origin. For our experiments, we have chosen two different pro- and anti-inflammatory immuneregulators. As pro-inflammatory immunoregulators, we used LPS and TNFα, whereas TGFβ and HGF were selected as anti-inflammatory immunoregulaors. CD40 expression in DCs is known to be upregulated following stimulation with pro-inflammatory mediators such as LPS and TNFα. In contrast, anti-inflammatory immunomodulators like HGF and TGFβ inhibit CD40 expression in DCs. Consistent with the previous reports, our data suggested that CD40 expression on the surface of immature BMDCs was increased following LPS or TNFα treatment. However, BMDCs pretreatment with HGF or TGFβ for 12 h inhibited this LPS- or TNFα-induced CD40 surface expression. These experimental observations suggested that pro- and anti-

inflmmatory factors differentially regulate CD40 expression in DCs. This prompted us to investigate whether these two distinct classes of immunoregulators engage any common transcription factor, which can both activate or inhibit CD40 expression. Such dual regulatory properties are known for RUNX transcription factors in the context of other target genes.

Strikingly, we have identified two putative RUNX binding sites in mouse *Cd40* promoter. Notably, these two RUNX-binding sequences are located in close vicinity of PU.1-binding site (etsB) in *Cd40* promoter. This raised the possible involvement of RUNX proteins in regulation of CD40 expression by pro- and anti-inflammatory agents in DCs. Our *in vitro* and *in vivo* experimental data demonstrated that mouse *Cd40* promoter was occupied by RUNX proteins at both binding sites. Among three members of RUNX family, RUNX1 and RUNX3 but not RUNX2 bind to *Cd40* promoter in DCs after treatment either with pro- or anti-inflammatory immunoregulators. We further determined the relative contribution of two RUNX binding sites in *Cd40* promoter activity in DCs. Although, each RUNX binding site contributed to *Cd40* promoter activity individually, the presence of both RUNX binding sequences was required for full promoter activation. Based on this information, we conclude that the RUNX binding sites are key regulatory elements of *Cd40* promoter. Our study involving RUNX1- and RUNX3- knockdown DCs shows that these RUNX proteins are required for activating and suppressing CD40 expression in response to pro- and anti-inflammatory stimuli, respectively. Collectively, our RUNX1 and RUNX3 knockdown data demonstrate that RUNX proteins play dual (i.e. both positive and negative) regulatory role in CD40 expression in DCs. Furthermore, we demonstrate that upon stimulation with soluble CD40 ligand; CD40 induced IL-12p70 secretion by DCs treated with pro-inflammatory mediators and that this secretion critically depends on RUNX-mediated regulation of CD40 expression.

On elucidating the upstream signaling events we found that pro- and anti-inflammatory factors induced distinct PI3K complexes and Akt isoforms. For instance, LPS and TNFα stimulation induced recruitment of p85α/p110β PI3K complex to their receptors; TLR4 and TNFR, respectively. This further induced downstream activation of Akt1. In contrast, HGF and TGFβ induced recruitment of PI3K complexes p85α/p110α and p85α/p110δ to c-MET and TGFR1, respectively. This further led to the activation of

Akt2 and Akt3. The induction of the p85α/p110β PI3K complex and activation of Akt1 by pro-inflammatory agents was found to correlate with the formation of RUNX/co-activator complex and upregulation of CD40 expression in DCs. Similarly, anti-inflammatory mediators induced the p85α/p110α and p85α/p110δ PI3K complexes and activated Akt2 and Akt3, which correlated with the recruitment of RUNX/co-repressor complexes to *Cd40* promoter leading to the suppression of CD40 expression in DCs. Our findings indicate that induction of distinct PI3K complexes and Akt isoforms plays a decisive role in differential regulation of CD40 expression in DCs by pro- and anti-inflammatory agents. We also provide evidence that activation of PI3K is required for binding of RUNX1 and RUNX3 to *Cd40* promoter. Our findings therefore suggest a novel role for the PI3K/Akt pathway in RUNX1- and RUNX3-regulated CD40 expression in DCs.

Overall, our study demonstrates that RUNX proteins act as potent regulators of CD40 expression in DCs. Furthermore, RUNX proteins and the upstream PI3K/Akt pathway play an essential role in differential regulation of CD40 expression in DCs by pro-inflammatory and anti-inflammatory mediators. Notably, aberrant expression of CD40 on DCs has been shown to influence many disease processes. Being a regulator of CD40, RUNX can provide a new therapeutic target because of its dual regulatory role in CD40 expression in DCs.