# Abstract

The receptor tyrosine kinase MET and its ligand the hepatocyte growth factor/scatter factor (HGF) control vital cellular processes such as survival, proliferation, differentiation and motility. HGF-induced MET signaling is critical in embryonic development and tissue regeneration at the adult stage. A dysregulation of HGF/MET pathway can drive tumorigenesis and metastasis. Ligand-mediated MET activation is dependent on CD44v6 isoforms, which are members of the CD44 family. The transmembrane CD44v6 protein has two functions: its extracellular part regulates the phosphorylation of MET and its cytoplasmic domain is involved in MET internalization and downstream signaling. MET has another ligand called Internalin B (InlB), found on *Listeria Monocytogenes*. In Listeriosis, InlB induces MET activation to infect host cells and this process also requires CD44v6.

Indirect binding studies between MET, CD44v6 and ligands HGF or InlB revealed the formation of a trimeric complex in tumors and several cell lines. In addition, HGF was found to only bind to cells expressing CD44v6 and a physical association was evidenced between the ectodomain of CD44v6 and HGF as well as InlB in solution. In contrast, no binding was identified between MET and CD44v6 in solution but the behavior of proteins in live cells may be different due to the constraints imposed by the plasma membrane. Furthermore, the mechanism by which CD44v6 and ligands of MET can associate in live cells is still unknown.

My PhD work consisted in characterizing the organization and dynamics of MET and CD44v6 to have a better understanding of MET activation on the plasma membrane. In this context, we observed a direct interaction between CD44v6 and MET upon induction of HGF and InlB in T-47D cells by Förster resonance energy transfer-fluorescence lifetime imaging microscopy (FRET-FLIM). In addition, fluorescence correlation spectroscopy (FCS) showed a reduced mobility of CD44v6 upon interaction with MET and HGF confirming the formation of an oligomeric complex of CD44v6-MET-HGF. Additionally, we monitored the oligomerization of CD44v6 by FCS and identified an increase in the dimer population of CD44v6 induced by HGF and InlB in T-47D cells containing only CD44v6.

Intriguingly, the binding of the two ligands also led to an increase in the mobility of the co-receptor CD44v6 in the membrane. Since cholesterol removal by methyl-β-cyclodexrin conducted a similar increase in CD44v6 mobility, we hypothesized that CD44v6 moved out of a lipid domain in order to cluster and interact with the ligand HGF. In addition, we investigated

the role of lipid domains in the activation process of MET in HeLa cells. In this aim, we targeted the major components of lipid domains such as cholesterol and sphingomyelin and assessed the phosphorylation of MET and downstream targets. None of the targeting strategies affected MET or ERK phosphorylation. This led us to hypothesize that MET activation is likely independent of lipid domains.

In conclusion, our data are in line with a model where CD44v6 would be recruited out of confined lipid domains to dimerize and bind to HGF or InlB. This complex would then present the ligands to MET proteins to form an oligomeric active complex for signaling.