Live-Cell Confocal Microscopy Imaging of Met Receptor Tyrosine Kinase and Actin Dynamics in Cell Migration

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Hepatocyte Growth Factor/Scatter Factor (HGF/SF) induces mitogenic, motogenic and morphogenic changes that are mediated by the Met receptor tyrosine kinase. Pathologically, Met-HGF/SF signaling is associated with tumorigenesis, invasiveness and metastasis.

We studied Met interaction and dynamics in live cells by confocal microscopy, using functionally active fluorescent tagged Met proteins. We characterized HGF/SF-induced membrane ruffling and studied Met dynamic localization in ruffling regions. We also analyzed Met dimerization and association with the actin cytoskeleton.

HGF/SF-induced ruffling was observed 5 minutes after treatment. Modification of the actin cytoskeleton, induced by jasplakinolide (jaspamide) or latrunculin A treatments, completely eliminated HGF/SF-induced membrane ruffling. High levels of Met, actin and the membrane marker (YFP-Mem) were observed in late-stage retracting ruffle regions, which formed long filopodia and shorter, spike-like membrane protrusions.

Fluorescence resonance energy transfer (FRET) analysis demonstrated an oscillation of Met-Met dimerization ($P<0.0001$) and Met-Actin association ($P<0.005$). Fluorescent recovery after photo-bleaching (FRAP) showed increased Met lateral mobility ($P<0.001$) after short-term HGF/SF stimulation. Continuous (24h) HGF/SF stimulation resulted in mobility arrest of Met but not significant internalization. Modification of the actin cytoskeleton attenuated Met lateral mobility by 20% ($P<0.01$).

We suggest that Met receptor activation induces a localized cascade of molecular and cellular alterations that lead to cell motility initiation. HGF/SF binding increases Met lateral mobility and induces its oscillated dimerization and association with actin.

Consequently, membrane ruffles are formed and several proteins accumulate in characteristic spiky membrane protrusions. Membrane ruffling and Met lateral mobility are dependent on the integrity of the actin cytoskeleton. We hypothesize that these membrane protrusions play a role in cellular chemosensing, possibly towards migration. These data may shed a light on the subcellular, spatial and temporal mechanism underlying the initiation of cell motility.