BIO-ID PROXIMITY MAPPING IDENTIFIES CEP63 AS A CENTROSOMAL TARGET OF HTLV-1 TAX ONCOPROTEIN

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Background:
One of the drivers of Adult T-cell Leukemia (ATL) onset is HTLV-1-induced genomic instability. In infected cells, a fraction of the viral oncoprotein Tax localizes at the centrosome, and expression of Tax is sufficient to trigger centrosome aberrations that could fuel genomic instability. However, the precise mechanisms underlying Tax-induced centrosome aberrations remain elusive, mainly because probing interactions at the centrosome using proteomic approaches has been challenging.

Methods:
We developed a proximity-dependent biotinylation assay (BioID) using a BirA-modified Tax. This new proteomic approach allows the in-situ labelling of proximity partners, which is particularly relevant to identify interactions in solubilization-resistant cell compartments. We then confirmed the interaction of Tax with new candidate partners using co-immunoprecipitation assays. Finally, we analyzed the consequences of these interactions on centrosomal defects by confocal microscopy in T cell lines.

Results:
The BioID screen allowed the identification of several proximity partners of Tax directly involved in centrosomal regulation, or indirectly modulating the cell cycle. These candidate partners included the centrosomal protein Cep63, a key regulator of centrosome duplication, cell cycle progression and DNA damage response. Co-immunoprecipitation assays confirmed the interaction of Tax with Cep63 and allowed us to map the domains involved. We further showed that the interaction with Tax induces a partial dispersion of Cep63 from the centrosome, which is correlated to alterations of the centriolar marker Centrin and the pericentriolar marker PCNT, and could be responsible for functional centrosomal defects.

Conclusion:
Altogether, these findings provide new insights into the Tax-induced centrosomal alterations that are thought to fuel the genomic instability and the onset of ATL.

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