

P012 The ESCRT-I subunit Tsg101 localises to the lumen of multivesicular endosomes

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The endosomal sorting complex required for transport (ESCRT) proteins are essential for receptor downregulation, which involves endocytosis of ubiquitinated growth factor receptors, maturation of endosomes into multivesicular endosomes (MVEs), fusion with lysosomes and subsequent degradation of the ubiquitinated cargo, resulting in the termination of its signaling. To date four different ESCRT complexes, ESCRT-0, -I, -II and -III have been characterised. Here we have determined the specific localisation of the ESCRT-I component Tsg101 within endosomes. Biochemical studies using cell fractionation showed that Tsg101 localises both to the cytosol and to membrane fractions and furthermore to the lumen of MVEs. This is in contrast with other ESCRT proteins, which localise to the limiting membrane of MVEs. The intraluminal localisation of Tsg101 was further verified by confocal microscopy investigating enlarged endosomes produced by expression of the GTPase deficient Rab5(Q79L)-mutant. The basal recruitment of Tsg101 to endosomal membranes is decreased in ESCRT-0 and ESCRT-II depleted cells, suggesting that these complexes are important for this process. Furthermore, Tsg101 localises to the lumen of MVEs only in cells cultured in medium containing 10% fetal bovine serum (FBS), while it is absent in MVEs of cells cultured in serum-depleted conditions. Since serum-starvation is associated with cell cycle arrest and reduced migration, we hypothesise that Tsg101 might be involved in the regulation of these processes by sorting proteins involved in cell adhesion into MVEs.