Endocytic mechanisms underpinning cancer resistance to a selective immunotherapy

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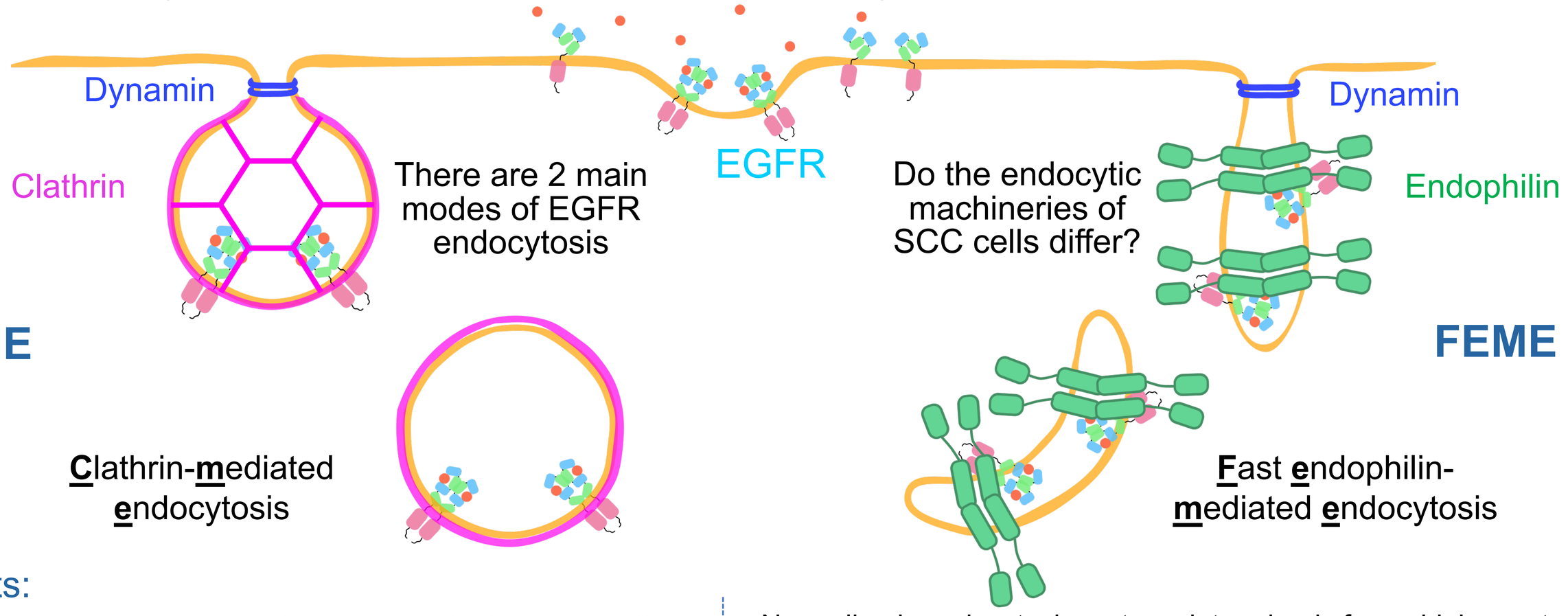
Background:

Head and neck squamous cell carcinoma (**HNSCC**) affects over 600,000 patients worldwide. 80% are resistant to an epidermal growth factor receptor (**EGFR**)-selective therapy, cetuximab. EGFR is involved in dysregulated cellular signalling and is internalised by at least 2 pathways: clathrin-mediated endocytosis (**CME**) and fast endophilin-mediated endocytosis (**FEME**).

We asked if the endocytic machineries in cetuximab-sensitive and -resistant SCC cell lines may prefer one of these endocytic pathways? Identifying differences between the endocytic machineries of cetuximab-sensitive and -resistant cancer cells may pinpoint novel biomarkers responsible for cetuximab-resistance and, in turn, advance options for HNSCC treatment.



Results:

KJD cells are uniquely cetuximab-resistant, and blotting reveals they have higher amounts of endophilin and dynamin over other SCC cell lines. Both CME and FEME involve dynamin. However, FEME is also dependent on endophilin.

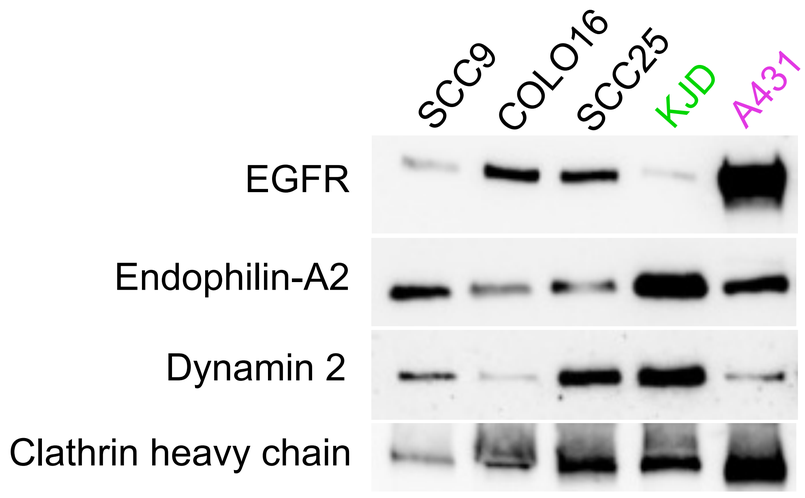


Figure 1. Relative expression of endocytic proteins in SCC lines.

Normalised endocytosis rates determined from high content imaging reveal that EGFR internalisation rates vary across SCC cell lines, and KJD (green) has the fastest rate. Could its significantly faster EGFR uptake be linked to trafficking through **fast** endophilin-mediated endocytosis (FEME)?

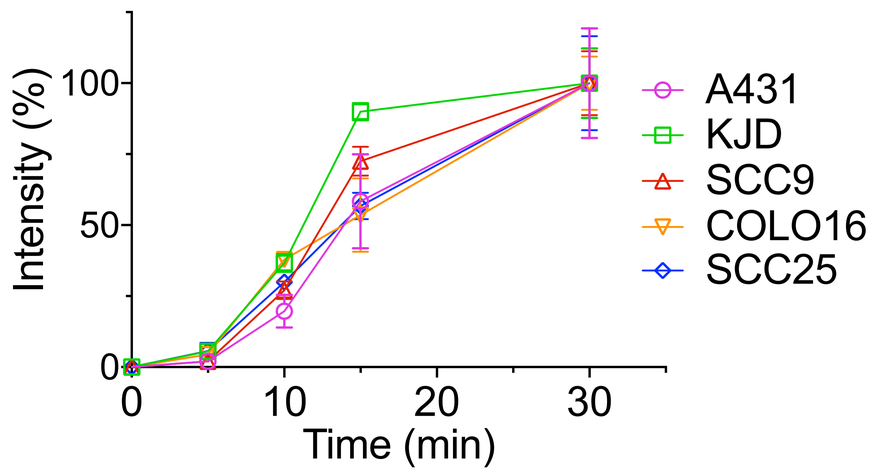


Figure 2. Rates of EGFR endocytosis differ in various SCC lines.

Receptor uptake through FEME begins when receptors are activated by high concentrations (≥ 10 ngmL^{-1}) of a cognate ligand at the plasma membrane of a cell. Identified by Boucrot *et al.* (2015), FEME is only observed at physiological temperatures (37°C in mammalian cells), and stimulation for 4 min is sufficient for the formation of endophilin-positive assemblies.

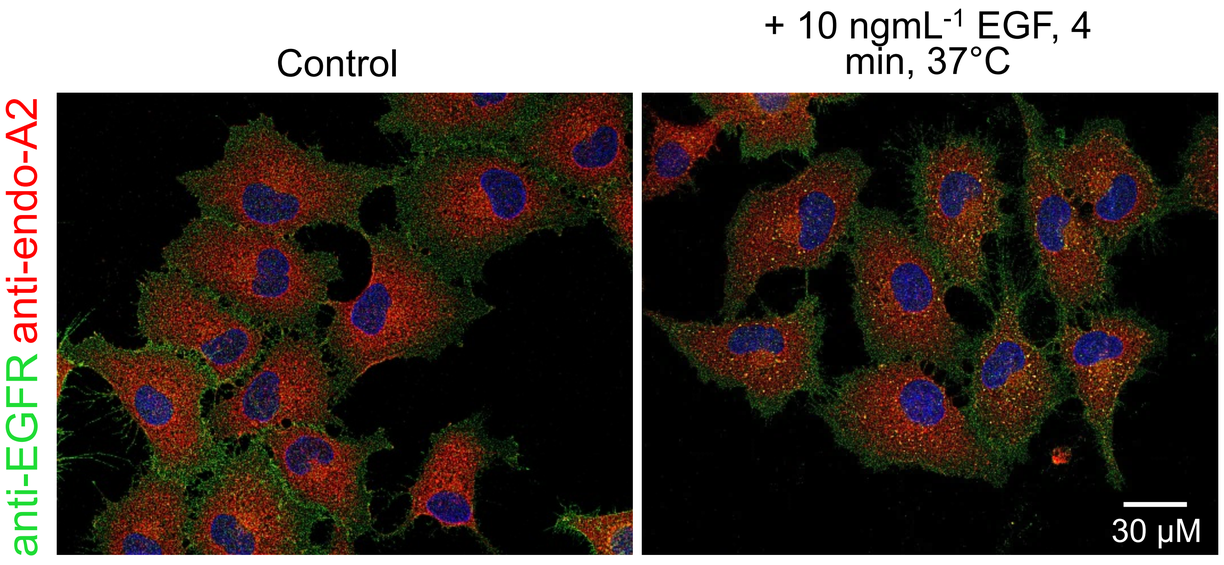


Figure 3. EGFR and endophilin colocalise in KJD cells (yellow, right).

If EGFR mainly traffics through FEME, uptake of EGF ligand should not be reduced by clathrin inhibition, as FEME does not depend on clathrin, unlike the well-established pathway CME. In KJD cells, reduced EGF uptake was observed upon dynamin inhibition, but not clathrin inhibition.

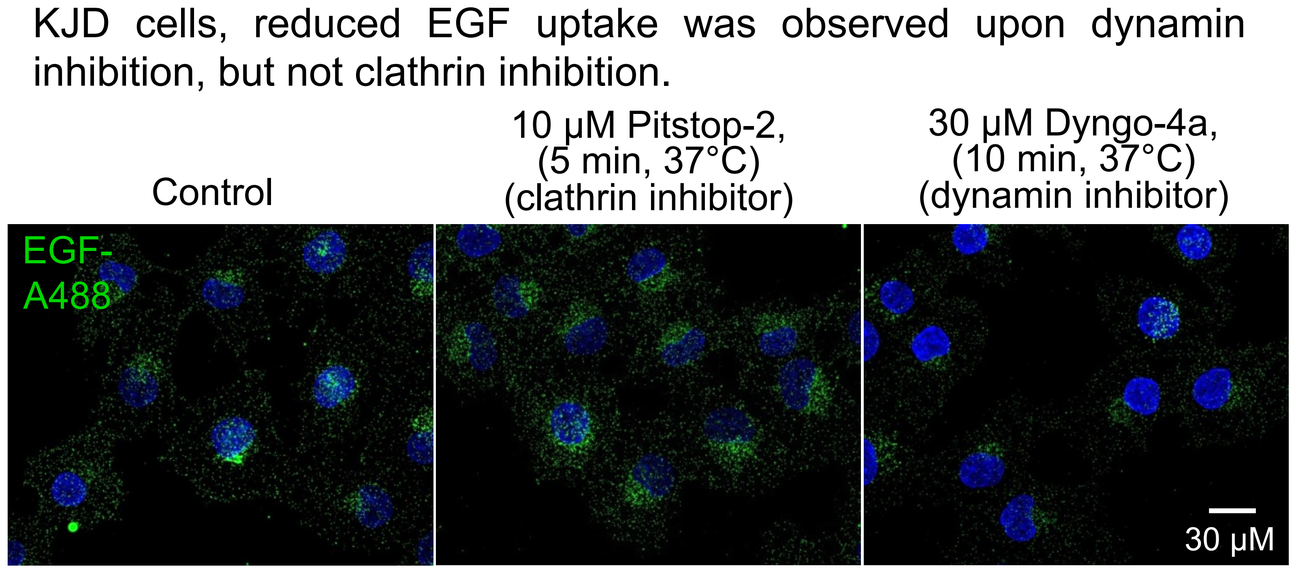


Figure 4. EGFR traffic in KJD cells is resistant to a clathrin inhibitor, sensitive to a dynamin inhibitor.

Conclusions and future directions

- Combined characteristics of KJD cells suggests EGFR traffics through a non-CME pathway, like FEME
- Downstream mechanisms of FEME remain unknown- proteomic studies are being pursued