Engineering synthetic adhesins and injectisomes in Escherichia coli K-12 to target mammalian cells for biomedical applications.

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One of the aims of synthetic biology is the design of microorganisms with novel capabilities that could be applied for the development of new vaccines, diagnostic sensors, and therapeutic interventions for major diseases such as cancer. This presentation will report the development of two important tools that enable us to precisely program *E. coli* K-12 bacteria to: 1) adhere to specific target cells; and 2) assemble filamentous injectisomes from type III secretion systems (T3SS) that act as "molecular syringes" for translocation of specific proteins into mammalian cells. Firstly, we designed synthetic adhesins based on the display of VHH domains on the bacterial cell surface with an outer membrane β-domain derived from intimin of enterohemorrhagic *E. coli* (EHEC). We generated synthetic adhesins against different antigen targets expressed on the surface of mammaliam cells and have demonstrated the specific adhesion of the engineered *E. coli* bacteria to the target mammalian cells using *in vitro* and *in vivo* models. Using mouse xenotransplants of human tumor cell lines expressing a target antigen, we demostrated that engineered *E. coli* bacteria colonize these tumors more efficiently at lower bacterial doses (Piñero-Lambea et al., 2015). Secondly, in order to express functional injectisomes in a non-pathogenic commensal *E. coli* strain (K-12), we reformatted the operons encoding the structural proteins and chaperones needed for the assembly of filamentous injectisomes from enteropathogenic *E. coli* (EPEC). Our synthetic operon constructs lack secreted effectors and regulatory elements found in EPEC. Five synthetic operons (sLEE1, sLEE2, sLEE3, sLEE4 and escD) were integrated into different sites of the chromosome of *E. coli* K-12 under the control of an inducible promoter (Ptac) using a marker-less strategy. We demonstrated that the resulting strain, named Synthetic Injector *E. coli* (SIEC), assembles functional injectisomes upon induction with IPTG able to translocate proteins into HeLa cells (Ruano-Gallego et al., 2015). Collectively, these results open the possibility to target specific mammalian cells with engineered *E. coli* bacteria and inject heterologous proteins of interest, such as antibody fragments, immunogens, enzymes, transcription factors, or toxins.

References

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