TITLE

ITA: Studio dell'organizzazione del recettore di membrana Her2 nei microdomini lipid raft in linee cellulari modello mediante nanostrutture di DNA e tecniche biofisiche avanzate.

ENG: Probing HER2 organization within lipid raft microdomains in model cell lines through DNA nanostructures and advanced biophysical methods.

DESCRIPTION OF THE PROJECT

HER2 overexpression, a hallmark of several cancers including breast cancer, poorly correlates with disease mechanisms and therapeutic response, highlighting the limitations of current biomarkers. Increasing evidence indicates that key determinants of HER2 signalling, such as receptor aggregation, **spatial organization at the nanoscale level**, and membrane context, are not captured by protein levels alone. In this framework, lipid rafts, specialized membrane microdomains enriched in sphingolipids and cholesterol, play a critical yet controversial role: while they can promote HER2 clustering, dimerization, and signalling, they may also restrict drug accessibility and alter receptor dynamics, leaving their overall impact on tumour progression and therapy response still unclear.

This project aims to elucidate the relationship between HER2 spatial organization and lipid raft composition by investigating the colocalization of HER2 clusters with key raft components, including GM1, cholesterol, and caveolin, in selected breast cancer cell models. To achieve this, we will employ RepliSeq, a **new DNA-based nanotechnology platform** capable of resolving protein proximity by using DNA nanoassemblies to translate membrane protein organization information into a digital DNA sequencing readout, enabling the analysis of membrane protein organization at the nanoscale. By applying this approach before and after treatment with targeted therapies, we will assess how lipid rafts influence HER2 homo- and hetero-oligomerization, as well as their role in therapeutic response and resistance. To further dissect these mechanisms, we will manipulate lipid raft integrity using chemical and genetic approaches, including cholesterol depletion and modulation of caveolin expression. In parallel, advanced membrane preparation techniques, such as giant plasma membrane vesicles (GPMVs) and mechanical cell stripping, will be used to access both extracellular and cytoplasmic sides of membrane proteins, enabling a comprehensive analysis of HER2 interactions. RepliSeq will be further integrated with **advanced biophysical techniques**, including Surface Plasmon Resonance (SPR) and infrared nanospectroscopy (nanoIR), to quantitatively characterize the binding dynamics, molecular interactions, and local biochemical composition of HER2-associated membrane nanodomains. High-resolution structural insights will be obtained through **cryo-correlative light and electron microscopy** (cryo-CLEM) combined with **focused ion beam scanning electron microscopy** (FIB-SEM) and electron tomography. This integrated approach will allow precise localization of HER2 clusters within membrane invaginations and correlation of their molecular composition with ultrastructural features.

By addressing key questions, such as whether HER2 clustering within lipid rafts drives tumour aggressiveness independently of expression levels, or whether differential confinement explains variability in response to therapy, this study aims to define a novel molecular signature based on HER2 spatial organization. Ultimately, this work will contribute to the development of more accurate predictive biomarkers and pave the way for personalized therapeutic strategies in HER2-positive breast cancer.

SUPERVISOR

Elena Ambrosetti

CONTACT DETAILS

Elena Ambrosetti
elena.ambrosetti@units.it
Università degli Studi di Trieste | University of Trieste
Dipartimento di Scienze della Vita | Department of Life Sciences
Ed. Q | Building Q – I piano | 1st floor, stanza 106 | room 106
Via Licio Giorgieri, 5 - 34127 Trieste – Italy