Meccanismi di resistenza agli inibitori dell'EGFR: blocco delle vie compensatorie di trasduzione del segnale e cross-talk con IL1

We previously reported that inflammatory cytokines (e.g. IL1, IL6 and IL8) can regulate cancer cell sensitivity to EGFR inhibition (Gelfo et al. 2016). Our working hypothesis is that interleukin receptors on the cell surface regulate EGFR signalling in normal and neoplastic cells.

Experimental plan

Task 1. Studying the functional relevance and achieving mechanistic insights in the cross-talk between EGFR and IL1R pathways

Validating the cross-talk between IL1R and EGFR signaling pathways. In order to investigate the crosstalk between EGFR and IL1R signaling pathways, we will generate cell clones stably expressing a secreted IL1R-Fc decoy. We will use HIEC (Human Intestinal Epithelial Cells), which are normal cells strictly dependent on EGF stimulus for survival and growth. We plan to stimulate HIEC naïve cells, or cells expressing the IL1R-Fc decoy, with IL1A alone or in combination with EGF. The activation of MAPK and AKT intracellular effector pathways will be evaluated, in order to reveal potential interactions between IL1R and EGFR signaling cascades. Furthermore, validation of EGFR addicted cell lines, will be performed, following the proposed scheme.

Phenotypic/functional analyses upon IL1-dependent regulation of EGFR signaling. The functional impact of IL1 stimulation or IL1R-blockade (by IL1R-Fc decoy) on the proliferation and migration of HIEC or colon cancer cells will be evaluated. Will be tested in cells forced to grow in suspension and forming colonspheres. Phenotypic analyses, such as the lumen status (filled or empty) of 3D spheres grown in matrigel, and invasive/irregular shape will be analysed, and coupled with the invasive or proliferative phenotype in adhesion BD matrigel coated transwells.

Functional Analyses of IL1R and EGFR activation.

Our preliminary data indicate that IL1R levels are upregulated upon cell treatment with EGFR inhibitors. In these conditions upregulation of cytokines is responsible for the acquisition of EMT markers and stem cell traits and ability to grow as colonspheres. Here we set to test the role of senescence in a sequential treatment with CTX, by evaluating specific markers, including BGal staining, HP1Gamma or heterochromatin foci and BrdU incorporation.

Task 2: Association of expression levels of NRG1/2 and ERBB receptors to osimertinib resistance in lung cancer patients

Experimental plan and preliminary data: Our preliminary data, obtained in cell lines, show that lung cancer cells treated with OSI, display increased amount of ERBB2 and ERBB3, both at mRNAs and protein levels. We didn't test for ERBB4, but it will be done as part of this Task. Moreover, OSI resistant cells secrete high amount of NRG1 in the medium (Fig. 2A). We will collect pre-treatment and post-treatment biopsies of patients, who relapsed under OSI. We will employ samples, available as clots or pellets from lung cancer patients, collected by transbronchial needle aspiration (TBNA), before starting the OSI treatment and upon acquisition of resistance (n = 9 patients per group; 2 experimental groups, namely cancer cells collected at the time of diagnosis, and cancer cells collected at the progression, calculated with t-test with two tails and effects size = 1.5, power = 80%, a err prob = 0,05). We will analyse the abundance of the mRNAs of ERBB receptors, NRG1-2 ligands and MET. This evaluation will make use of the "*one-Step RT-ddPCR Advanced Kit*", which allows precise RNA target quantification by *Droplet Digital PCR (ddPCR)* in presence of scanty material [24]. This is a relatively newborn technology that has been used for several applications, such as rare DNA mutation detection, copy number variation analysis and absolute nucleic acid quantification also in paraffine fixed samples. The technology is currently available at our Department. Archival

pathological material derived from pre- and post-treatment biopsy is also available for additional studies.

Task 2.2 Evaluation of ERBB3 and MET by IHC in OSI resistant lung cancer patients.

Because of the secreted nature of NRG1 ligands, it will be not possible to perform IHC on patient slides. Indeed, most likely these ligands will be washed out during the IHC procedure. On the other hand, ERBB3 and MET protein evaluation relies on calibrated protocols running on the standard Ventana automated system of the pathology Unit. Thus, HER3 and MET will be evaluated in patients' samples collected before and after the insurgence of the resistance (n = 9 patients per group; 2 experimental groups, calculated with t-test with two tails and effects size = 1.5, power = 80%, a err prob = 0,05). Our collaborator, Prof. Fiorentino, head of the Pathology Unit of Ospedale Maggiore-Sant'Orsola IRCCS provided a letter of collaboration for this issue.