Phenotypic characterization of a drug tolerant state induced by targeted therapy in EGFR-mutated lung cancer cells.

Sarah FIGAROL 1,2, Olivier CALVAYRAC 1, Julien MAZIERES 1,4, Anne PRADINES 1,3, Gilles FAVRE 1,2,3.

1Cancer Research Center of Toulouse, U1037 INSERM, Toulouse, FRANCE.
2University Paul Sabatier, Toulouse, FRANCE.
3Institut Claudius Regaud, Institut Universitaire du Cancer de Toulouse-Oncoopole, Laboratory of medical biology and oncogenetics, Toulouse, FRANCE.
4CHU Toulouse, IUCT-Rangueil-Larrey, Toulouse, FRANCE.

Purpose:
While EGFR-mutated NSCLC (Non-Small-Cell Lung Cancer) patients benefit from EGFR-Tyrosine Kinase Inhibitors (TKI), most of them relapse due to the apparition of resistances. Recent evidences suggest that these resistances may arise from a small population of non-mutated drug-tolerant cells originally called “Drug-Tolerant Persisters” (DTPs) that initially resist the treatment by entering a slow-to-non cycling state. We recently reported a role of the RAS-related GTPase RHOB in the resistance to EGFR-TKI in EGFR-mutated lung cancer patients, suggesting that RHOB pathway could be a determinant of this DTP state acquisition. Our objective is to understand how the adaptive response of EGFR-mutated NSCLC cells soon after EGFR-TKI treatment leads to secondary resistance, by extensively characterize phenotypic changes associated with EGFR-TKI-induced DTP formation in a panel of EGFR-mutated cell lines.

Experimental Design:
EGFR-mutated PC-9, HCC827, HCC4006, H3255, and HCC2935 lung cell lines and PC9 KO RHOB cells were treated with EGFR-TKI erlotinib at 1μM and phenotypic characterization of the DTP state as well as time-lapse microscopy experiments were performed.

Results:
Time-lapse microscopy experiments revealed that this so-called DTP state appeared to be more complex than as it was previously reported in PC9 cell line. Indeed, we observed a high variability intra- and inter- cell lines for cell division rate/cell arrest, cell shape rearrangement and kinetics of resistant clones’ onset. Several DTPs showed enlarged shape, harbored increased F-actin fibers and displayed senescent-associated features, along with variations in the expression of several EMT markers (ie Vimentin or E-cadherin). As previously reported in the PC9 cell line, we also observed chromatin state modifications and increased sensitivity to HDAC inhibitors although this was highly cell-line specific, suggesting that different mechanisms might be involved in the DTP state acquisition. Consistent with a role of RHOB in DTP survival, the expression and activity of this GTPase were increased in drug-tolerant cells, and RHOB-KO PC9 cells showed a decreased proportion of DTPs compared to WT cells.

Conclusion:
The observed variability among cell-lines suggests that DTP state acquisition could be relied on distinct mechanisms. Characterization of these processes will help to better understand the adaptive resistance to EGFR-TKI in NSCLC to bring new therapeutic approaches to eliminate reservoir of drug-tolerant cells and to prevent emergence of resistance mutations occurring during the non-cycling state of tumor cells.

References:
Sharma et al., Cell, 2010; Hata et al., Nature Medecine, 2016; Calvayrac et al., EMBO Molecular Medecine, 2017.