Abstract

Non-small cell lung cancer (NSCLC) has been recognized as one of the most common and deadliest cancers worldwide in both genders. Even though in the last two decades, advances of NSCLC treatment have gained significant improvement, the prognosis of NSCLC remains poor with the figure of 5-year survival being roughly 20%. Comparable to other malignant entities, the strong dependence of NSCLC on tumor microenvironment (TME) has achieved great attention as the crosstalk between tumor cells and supportive cells would define the outcome of patients. Among a variety of cellular and soluble components, tumor-associated macrophages (TAMs) have been proven to be a potential target since this innate immune cell type is one of the most abundant components which not only apply the effects on the progression of tumor cells but also interact with other immune cells to fully form the TME landscape.

LYN kinase is a member of SRC family kinases (SFKs), which has been shown to play essential roles in regulating supportive capacity of TAMs in context of chronic lymphocytic leukemia (CLL). In detail, in the coculture system of CLL cells with macrophages, the removal of LYN in macrophages was able to diminish the viability of CLL cells. Taking the advantage of available LYN-ko THP1 macrophages, we performed this project, aiming to clarify whether LYN-ko macrophages could show their less supportive capacity to cancer cell in context of NSCLC. A panel of NSCLC cell lines were cocultured with Wildtype (Wt) or LYN-ko macrophages, followed by an array of functional assays.

Importantly, we could prove that LYN kinase showed upregulated expression in macrophages upon the supply with conditioned medium (CM) from NSCLC cell line. This result was further reinforced with the observation that the LYN kinase expression in NSCLC samples was higher in macrophages in tumor-proximal regions compared to macrophages in tumor-distal regions at both RNA and protein levels. Notably, despite the varied response of different NSCLC cell lines to macrophages via CM, we could show that LYN deletion in macrophages resulted in significantly reduced proliferation and invasion/migration capacity of NSCLC cell lines via CM transferring. In context of direct coculture, by marking the cells with different fluorescent proteins, the number of NSCLC cells were detected to be significantly lower in coculture with LYN-ko THP1 macrophages compared to Wt counterpart. The same effects have been recaptured with cell lines from murine models. Translationally, THP1 macrophage model failed to indicate any meaningful effects when tyrosine kinase inhibitors (TKIs) were applied to inhibit activity of tyrosine kinase such as LYN. In contrary, with primary cell model, particularly PBMC-derived M2-macrophages (Peripheral blood -derived monocytes), TKIs such as dasatinib and bafetinib could show the ability to dampen supportive capacity of macrophages, in line with the results obtained from experiments using gene-editing THP1 cells.

To elucidate the mechanism, a multiomics experiment including transcriptome and proteome was performed on NSCLC cells following exposure to macrophage-derived CM. The overlapping between cell lines indicated a landscape in which down-regulation of mitochondrial translation and activity were observed in cells treated with LYN-ko THP1 macrophage-derived CM. This phenomenon could serve as a perfect explanation since mitochondria is not only the cellular power supply but also regulate cellular processes via multiple pathways such as the production of pro-tumor reactive oxygen species (ROS), a byproduct of oxidative phosphorylation (OXPHOS).

Cumulatively, the data achieved in this project indicates the key functions of LYN kinase in macrophage to maintain its support to the progression of NSCLC cells, which would be further validated with transplantation experiment in murine model.