Cohort of NSCLC patients to study expression of SFK members: bioinformatic analysis

The Cancer Genome Atlas (TCGA) database was used to study mRNA expression of the SFK members YES1, SRC, FYN and LYN in non-tumor lung tissue (n=109) and lung cancer specimens (n=1016). Comparisons between non-malignant and malignant samples were performed with the U-Mann Whitney test. For survival analyses, the publicly available bioinformatic tool Km plotter (https://kmplot.com/analysis) was used following previously published recommendations [1] (see below, in this document, for these references). This tool includes datasets from TCGA, Gene Expression Omnibus (GEO) and the Cancer Biomedical Informatics Grid (caBIG). Criteria for patient's selection were as follows: NSCLC patients from any stage or histological type, who did not receive any treatment. Taking into account these criteria, a total number of 227 patients were included in the Kaplan-Meier analysis. The median expression was set as cut-off value and overall survival was considered up to 60 months.

Tumor microenvironment profiling in NSCLC patients with CIBERSORT

TCGA-LUAD (155 cases) and TCGA-LUSC (248 cases) RNAseq gene expression data with standard annotation were uploaded to the CIBERSORT web portal (http://cibersort.stanford.edu/), and the algorithm was run using the LM22 signature and 1000 permutations [2]. Only the cases with a CIBERSORT output of $p < 0.01$, indicating that the inferred fractions of immune cell populations produced by CIBERSORT were accurate, were selected for further analyses [3,4]. Inferred T regulatory cell and CD8 T cell fractions for each TCGA patient, were matched with their corresponding YES1 expression.

Subsequent analyses were carried out by stratifying YES1 expression in high (upper quartile (Q4)) or low (Q1+Q2+Q3).

Cytotoxicity assay

UN680 (500 cells per well) and 393P (500 cells per well) were seeded in 24 well plates and dasatinib was added 24h later at different doses. Cell proliferation was determined 5 days after dasatinib addition by staining the cells with crystal violet (0.25% in methanol:$H_2$0 at dilution 1:1) and calculating the percentage of the area stained per well with the software Image J.

Quantitative real time PCR (qPCR) and Western blotting

RNA extraction, qPCR and Western blot methods were performed as previously described [5]. GAPDH was used as endogenous gene for qPCR. Primer sequences for the genes studied are shown in Supplementary Table 3. For Western blotting, the primary antibodies are specified in Supplementary Table 4.

YES1 knockdown in the 393P cell line

shRNAs targeting murine YES1 and a control shRNA were purchased from Sigma (TRC23614). Lentiviral particles were produced by transfection of HEK293T cells with 2 µg of the plasmid of interest in the presence of lentiviral packaging plasmids and X-tremeGENE HP DNA Transfection reagent (Sigma). Then, viruses were collected and 393P cells were infected with 300 µL of lentivirus and 8 µg/mL of polybrene (Sigma). Cells were selected with 5µg/mL of puromycin for a week. Finally, YES1 knockdown was checked by western blotting.

IFN-γ-based enzyme-linked immunospot (ELISpot)

Splenocytes ($7x10^5$) were cultured for 24h in the presence of $7x10^4$ irradiated 393P cells in 96 well-ELIIP plates (Millipore) previously coated with anti-IFN-γ antibody (clone AN-18; 1:250; Mabtech). Then, wells were washed and incubated with a biotinylated anti-IFN-γ antibody (clone R4-6A2; Mabtech) followed by streptavidin-ALP (Mabtech) and BCIP/NBT substrate. IFNγ spots were counted using a CTL ImmunoSpot S6 micro-analyzer (Cellular Technology).

References:

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