Breast cancer induces tolerogenic state of healthy activated CD4+ lymphocytes, characterized by reduced PI3K, NFκB, JAK-STAT, Notch, and increased TGFβpathway activity

Anja van de Stolpe
Philips Research, The Netherlands

Cancer cells can induce a state of immunotolerance, which may be reversed by checkpoint blocker immunotherapy. Prediction of immunotherapy response remains a challenge. CD4+ lymphocytes are important for activating the adaptive immune-response, while conversion to immune-suppressed state impairs the anti-cancer immune-response. Lymphocyte function is controlled by a number of signaling pathways. We developed tests to quantitatively measure activity of signaling pathways (e.g. Hedgehog, Wnt, TGFβ, Notch, NFκB, PI3K, JAK-STAT 1/2 and 3, and MAPK) based on Bayesian model inference of activity from measurements (microarray, qPCR) of mRNA levels of target genes of the transcription factor associated with the respective signalling pathway[1],[2],[3],[4],[5],[6]. Tests were biologically validated on individual cell/tissue samples, including immune cells and can be used to characterize functional activity status of immune cell types. The cellular mechanism underlying breast cancer-induced immunosuppression of CD4+ lymphocytes was investigated.

Method: Generation of Affymetrix expression microarray data has been described [7]. In brief, dissected breast cancer tissue fragments from fresh surgical specimens were mechanically dissociated in X-VIVO-20 (SN). Following standard activation, healthy donor CD4+ lymphocytes were incubated with SN. Signaling pathway activities were measured on Affymetrix data from the CD4+ lymphocyte samples.

Results: CD4+ lymphocyte activation resulted in induction of PI3K, NFkB, JAK-STAT1/2, JAK-STAT3, and decrease of TGFβ pathway activities. Incubation with cancer SN only reduced activity of PI3K, NFkB, JAK-STAT1/2, JAK-STAT3 pathways, while increasing TGFβ pathway activity, in activated lymphocytes, but. Conclusion: A soluble factor from breast cancer tissue induces immunotolerance in CD4+ lymphocytes, by increasing TGFβ pathway activity, and reducing activity of effector immune pathways. Investigation as to the nature of the soluble factor is in progress. Signaling pathway assays can quantitatively measure the functional immune activity state of CD4+ lymphocytes. Envisioned application is in prediction and monitoring of immunotherapy response and identification of novel drug targets to reverse cancer-induced immunosuppression.

anja.van.de.stolpe@philips.com