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TWIST1 KNOCKDOWN ELUCIDATES THE REGULATION OF TH17-LIKE RESPONSE IN HER2 BREAST CANCER SUBTYPE

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Introduction and objectives: Breast cancer (BC) is a heterogeneous disease, composed by multiple subtypes with different molecular characteristics and clinical outcomes. In Brazil, this neoplasia is the first cause of cancer death in women, mainly due to late diagnosis, when the possibility of developing metastasis is improved. The metastatic process depends on the expression of transcription factors (TFs) related to epithelial-mesenchymal transition (EMT). Among these factors, Twist1 is described to be the master regulator of EMT in BC, although its role in BC subtypes remains unclear. The aim of our study is to investigate the role of Twist1 in intrinsic molecular subtypes of breast cancer. Material and methods: We evaluated the mRNA levels for NF-κB, Twist, Slug, and Sip1 on 46 breast tumor samples. We also performed Kaplan-Meier curves to associate gene expression to survival. Then, we silenced Twist1 expression in HCC-1954 (HER2) cells using shRNA-approach, confirmed the knockdown by RT-qPCR and immunoblotting, and subsequently performed a microarray analysis by GeneChip human exon array, whose findings were analyzed on Metacore software. We confirmed some altered genes expression using RT-qPCR. Finally, we examined IL-17 signaling members and its downstream targets by flow cytometry, immunoblotting and ELISA. Results and conclusion: In BC samples, we observed that Triple-negative group expressed more Slug and Sip1 and, interestingly, Twist1 was overexpressed in HER2 group. Kaplan Meier showed higher probability of death for those patients who expressed high levels of NF-κB and Twist. The TWIST1 knockdown in HER2 cells provoked profound molecular alterations, since 141 genes were up-regulated and 190 down-regulated. In silico analysis revealed numerous correlations between Twist1 with important biological processes and signaling pathways, such as EMT via TGF-β/SMADs, extracellular matrix remodeling, Th17 signaling, among others. Interleukin (IL) -17 signaling was examined through the expression of IL-17RA and Act1 proteins, which act to trigger this signaling together with IL-6 and IL-8 levels, which are targets of this signaling. Both results reported, consistently, that Twist1 plays an important role in activating a Th17 profile in HER2 BC context. Finally, our findings may contribute for a better understanding of Twist1 role in Her2 breast cancer subtype and point out Twist1 as potential target for the development of new therapies.