Functional characterization of pi3k c2 domain mutations detected in breast cancer circulating tumor cells and metastatic cells

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Abstract

Background: In breast cancer, over one third of all patients harbor a somatic mutation in the PIK3CA gene, encoding the catalytic subunit of the phosphatidylinositol 3-kinase (PI3K) in their tumor cells. Circulating tumor cells (CTCs) are cells shed from the primary tumor into the blood stream. Recently, the long-term stable breast cancer CTC-ITB-01 cell line with tumorigenic and metastatic capacity was established from liquid biopsy derived cells. The oncogenic hotspot PIK3CA mutation H1047R (kinase domain) was detected in the primary tumor, CTCs and metastasis of the same patient. Other PIK3CA mutations located within the C2 domain (E418K and E453K) were detected in the CTCs and the vaginal metastasis but not in the primary tumor. The goal of our study was to functionally characterize the impact of the E418K and E453K mutations within the C2 domain.

Methods: PIK3CA mutations E418K, E453K, H1047R were generated by site-directed mutagenesis and stably overexpressed in breast cancer cells by lentiviral transduction. The impact of PIK3CA mutations on signal transduction, membrane localization and biological processes was studied by western blot analysis and live cell imaging. Structural modeling was conducted in Pymol.

Results: Western blot analysis of human MDA-MB-231, MCF-7 and T47D breast cancer cells stably overexpressing either the PIK3CA wildtype (WT) or one of the E418K, E453K or H1047R mutants revealed a significant increase in AKT phosphorylation in both C2 mutants (E418K and E453K) and the kinase domain mutant H1047R. Functional analysis showed a significantly increased proliferation of MDA-MB-231 cells overexpressing the E453K and H1047R mutants. Interestingly, invasion and chemotaxis were only enhanced in the MDA-MB-231 cells overexpressing the C2 domain mutants, i.e. E418K and E453K. In addition, membrane localization of the two C2 domain mutants was increased. Structural modeling of the E453K mutation suggests a disruption of the interaction between the negative regulatory domain of the p85α subunit and the p110α catalytic subunit as a potential mechanism leading to the observed activation of PI3K/AKT/mTOR signaling.

Conclusions: Our results demonstrate that PIK3CA C2 domain mutations activate PI3K downstream AKT signaling and can increase proliferation, migration and invasion after stable lentiviral transduction.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.