c-Src signaling in triple negative breast cancer cells: role of Cyr61

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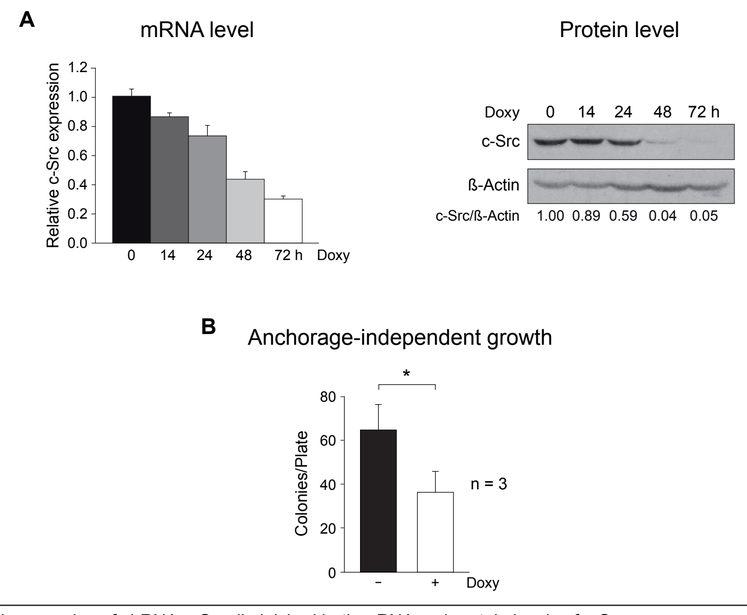
Abstract

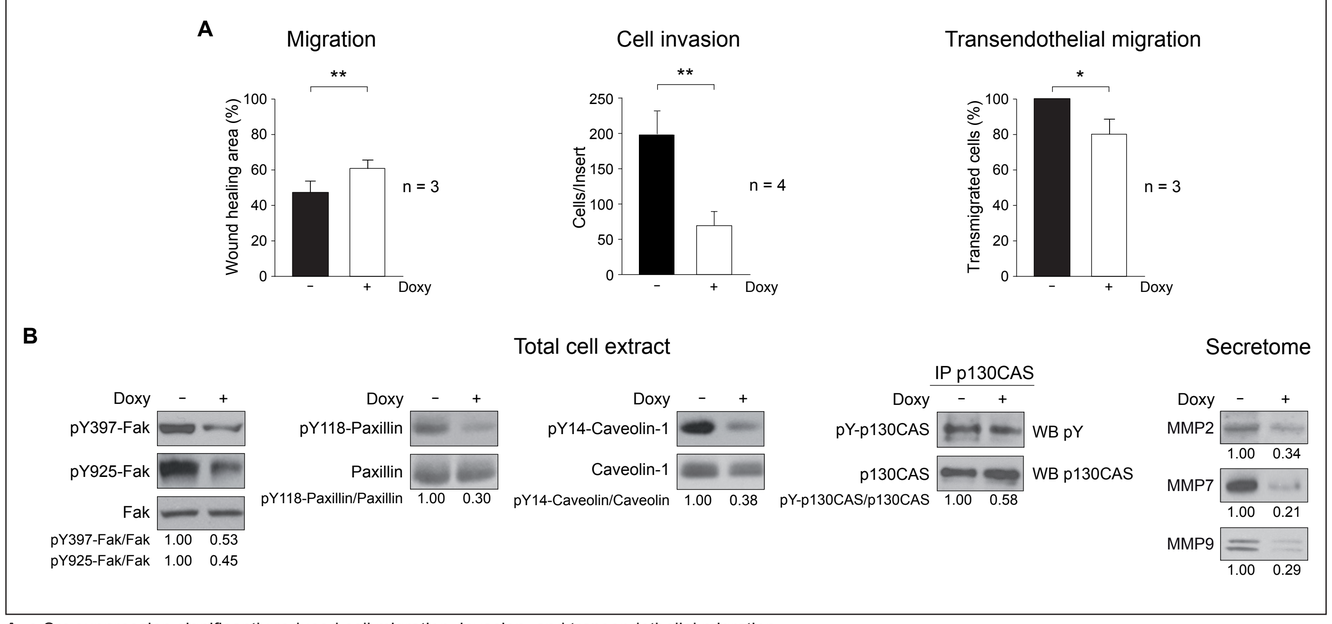
SFKs are involved in tumorigenesis and metastasis. Here we analyzed c-Src contribution to initial steps of metastasis by tetracycline-dependent expression of a specific shRNA-c-Src, which suppressed c-Src mRNA and protein levels in metastatic MDA-MB-231 cells. c-Src suppression did not alter cell proliferation or survival, but it significantly reduced anchorage-independent growth. Concomitantly with diminished tyrosine-phosphorylation/activation of Fak, caveolin-1, paxillin and p130CAS, c-Src depletion also inhibited cellular migration, invasion and transendothelial migration. Quantitative proteomic analyses of the secretome showed that Cyr61 levels, which were detected in the exosomal fraction, were diminished upon shRNA-c-Src expression. In contrast, Cyr61 expression was unaltered inside cells. Cyr61 partially colocalized with cis-Golgi gp74 marker and with exosomal marker CD63, but c-Src depletion did not alter their cellular distribution. In SUM159PT cells, transient c-Src suppression also reduced secreted exosomal Cyr61 levels. Furthermore, conditional expression of a c-Src dominant negative mutant (SrcDN, c-Src-K295M/Y527F) in MDA-MB-231 and in SUM159PT diminished secreted Cyr61 as well. Cyr61 transient suppression in MDA-MB-231 inhibited invasion and transendothelial migration. Finally, in both MDA-MB-231 and SUM159PT, a neutralizing Cyr61 antibody restrained migration. Collectively, these results suggest that c-Src regulates secreted proteins, including the exosomal Cyr61, which are involved in modulating the metastatic potential of triple negative breast cancer cells. (Oncotarget. 2015 May 30;6(15):13520-38.)

Figure 1
Cell proliferation and anchorage-independent growth
(MDA-MB-231)



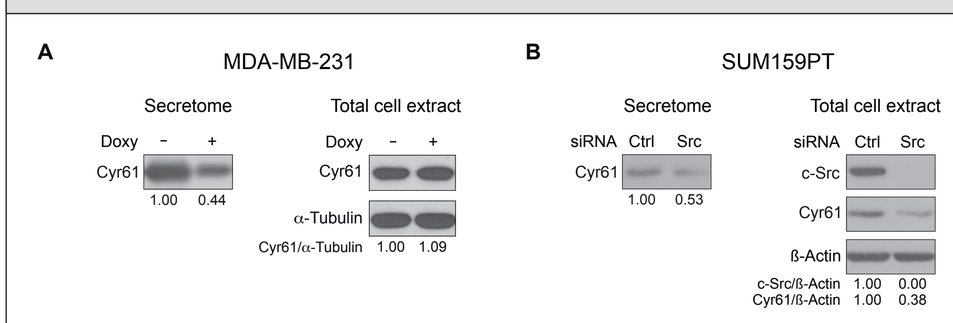
A. Conditional expression of shRNA-c-Src diminished both mRNA and protein levels of c-Src.
B. c-Src suppression reduced anchorage-independent growth, while it did not alter cell proliferation and viability (data not shown).

Figure 2
Migration and invasion
(MDA-MB-231)



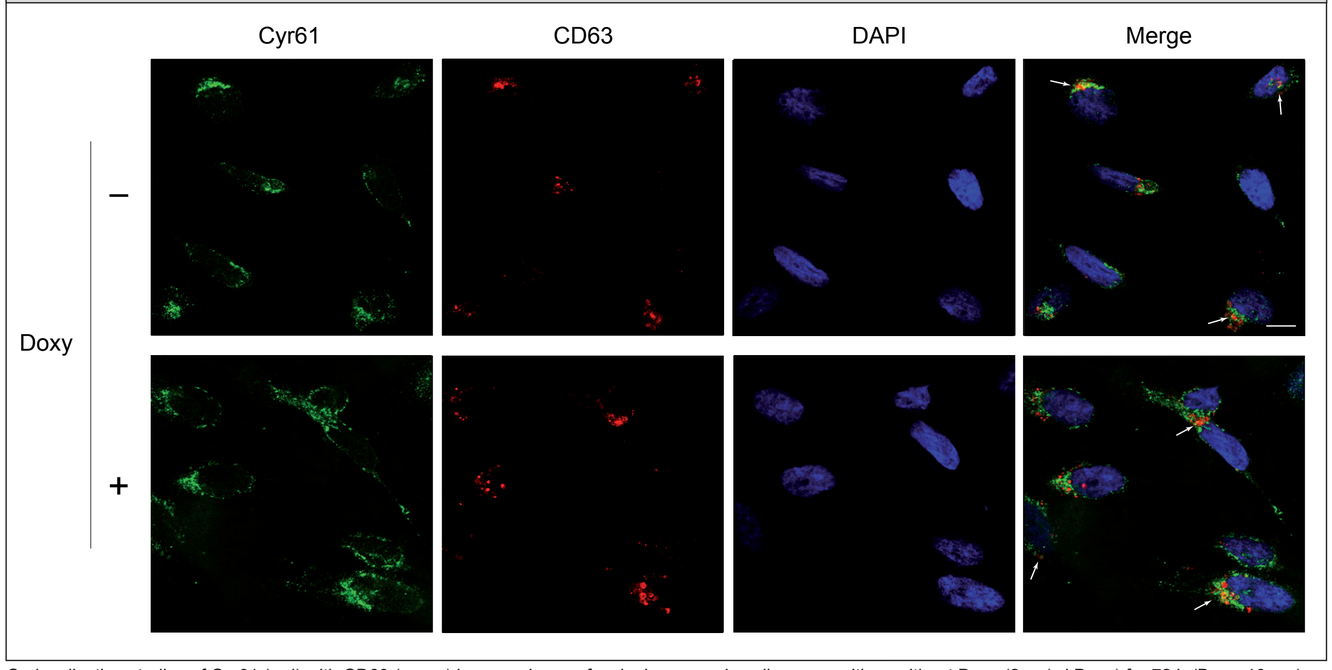
A. c-Src suppression significantly reduced cell migration, invasion, and transendothelial migration.
B. c-Src depletion decreased the degree of tyrosine-phosphorylation/activation of the focal adhesion proteins Fak, Paxillin, Caveolin 1, and p130CAS, as well as the levels of secreted metalloproteinases MMP2, MMP7, and MMP9.

Figure 3
Suppression of c-Src reduces Cyr61



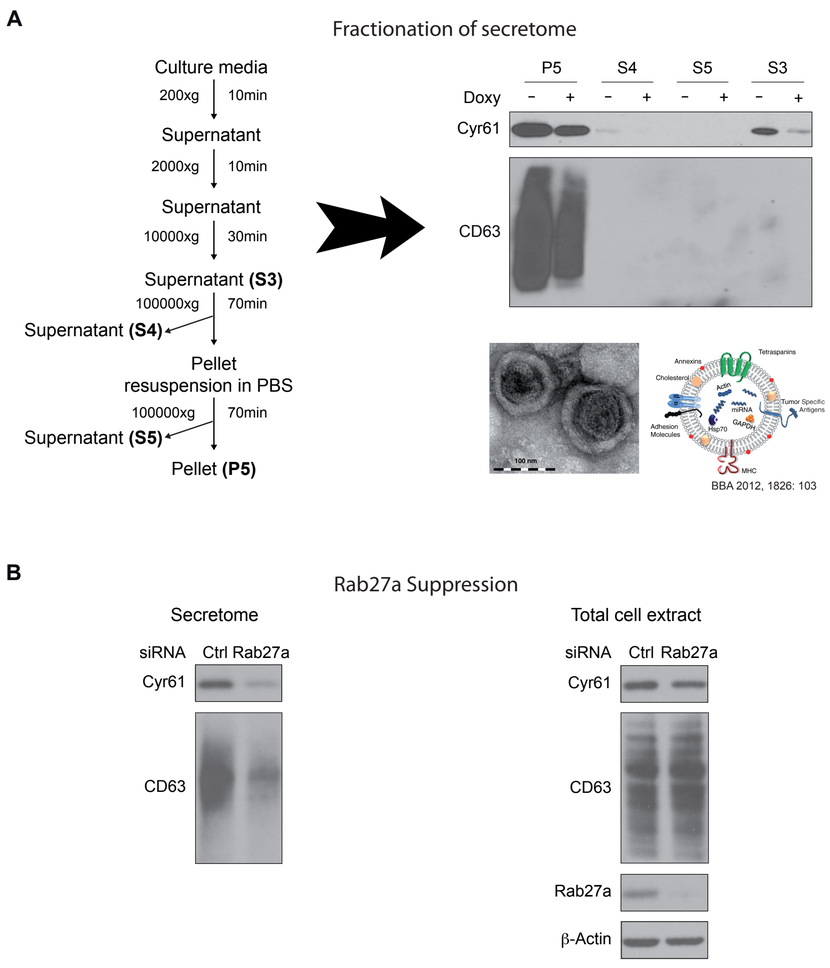
A. In MDA-MB-231 cells, c-Src suppression reduced the levels of Cyr61 (Cystein-Rich-61) in the secretome, but not Cyr61 intracellular levels of mRNA (data not shown) and protein.
B. In SUM159PT cells, c-Src depletion diminished the secretome and intracellular levels of Cyr61.

Figure 4
Colocalization Cyr61 with CD63
(MDA-MB-231)



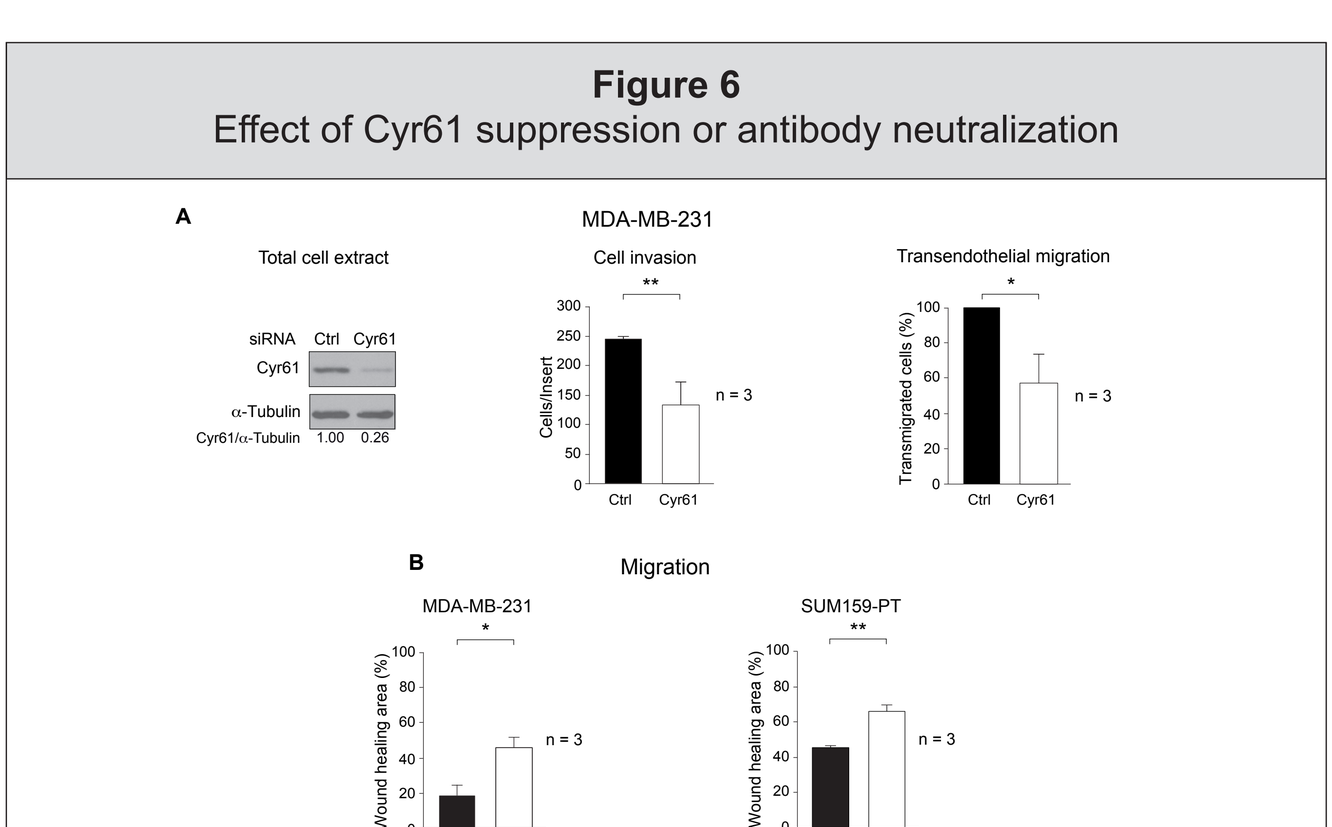
Co-localization studies of Cyr61 (red) with CD63 (green) by scanning confocal microscopy in cells grown with or without Doxy (2 μg/ml Doxy) for 72 h (Bar = 10 μm). White arrows indicate co-localization. Pearson's coefficients for Cyr61 co-localization with CD63 was 0.54, in absence of Doxy, and 0.51, in presence of Doxy.

Figure 5
Analyses of cellular and secretome Cyr61 distribution
(MDA-MB-231)



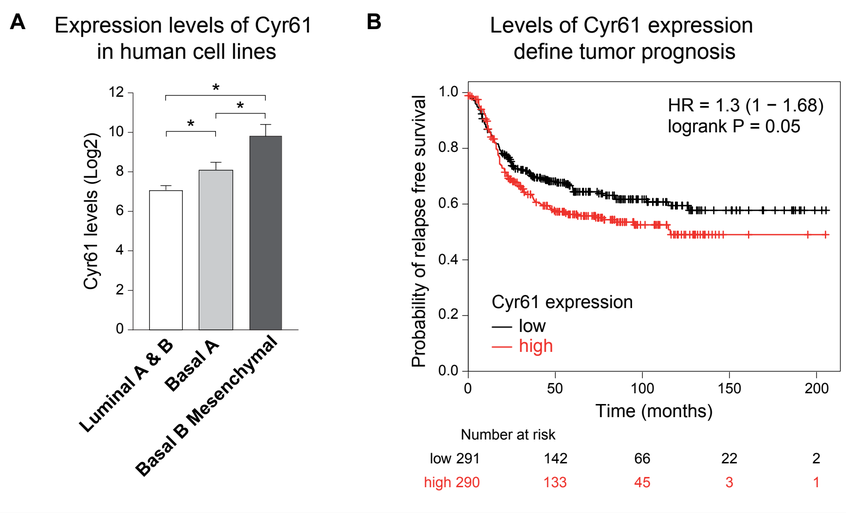
A. Scheme of secretome fractionation by differential centrifugation. Cyr61 was mainly in the exosomal fraction (P5), and c-Src suppression significantly reduced Cyr61 levels in this fraction, while the levels of the exosomal marker CD63 remained unaltered in P5. Schematic representation of exosomes.
B. Blocking the secretory pathway of exosomes by deleting Rab27a, a small GTPase involved in exosomal secretion, significantly reduced the levels of both Cyr61 and CD63 in secretome, supporting the localization of Cyr61 in exosomes.

Figure 6
Effect of Cyr61 suppression or antibody neutralization



A. Suppression of Cyr61 in MDA-MB-231 cells significantly reduced cell invasion, and transendothelial migration, to a similar extend as c-Src suppression (Figure 2).
B. Antibody neutralization of Cyr61 significantly inhibited cell migration of MDA-MB-231 and SUM159PT.

Figure 7
Expression of Cyr61 in breast cancer



A. Analysis of databases for Cyr61 expression in 51 breast cancer cell lines showed a positive correlation between high levels of Cyr61 expression and the most aggressive phenotypes.
B. Database analyses of 581 human samples of basal tumors showed that high levels of Cyr61 expression were significantly associated with poor prognosis.

Conclusions

1. c-Src participates in the anchorage-independent growth in TNBCs.

2. c-Src is involved in the regulation of cell migration, invasion, and transendothelial migration, modulating the levels of both tyrosine-phosphorylation/activation of focal adhesion proteins, and MMPs in the secretome.

3. The partial intracellular colocalization of Cyr61 and CD63, the Cyr61 presence in the exosomal fraction, and its secretion dependent on Rab27a, indicate that Cyr61 is mainly secreted in exosomes.

4. Conditional suppression of c-Src reduces Cyr61 levels in the secretome and in the exosomes. In turns, Cyr61 suppression mimics the cellular effects of c-Src depletion, as it does Cyr61 antibody neutralization.

5. Cyr61 is, at least in part, a mediator of c-Src in the initial steps of metastatic processes of TNBC cells.