CX32 CELLULAR LOCALIZATION IS RELATED TO EPITHELIAL TO MESENCHYMAL TRANSITION IN BREAST CELLS

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Connexins (Cx) play both gap junction-related and -independent roles in cells, and their localization is essential for their function in cellular processes. Besides membrane localization, connexins can also be localized to the cytoplasm and nucleus, especially in cancer cells. The differential localization of connexins including Cx32 was observed in different stages of cancers. Cx32 was upregulated and observed in cytoplasms of cells in lymph-node metastasis of breast cancer samples compared to primary tumors. However, the significance of the increase in Cx32 expression and alteration of Cx32 cellular localization in epithelial-to-mesenchymal transition (EMT) is not known. To determine if Cx32 overexpression and/or localization over one week would induce the EMT process we first examined the cellular localization of Cx32 in MCF10A and MDA-MB-231 cells at different time points using Western blot and RT-PCR as well as immunostaining with confocal microscopy. Then, we correlated the changes of Cx32 expression and localization with EMT marker expression. We showed that Cx32 had altered cellular localization and Cx32 overexpression increased Slug levels while it reduced E-cadherin and Snail expression in MDA-MB-231 for 7 days. In contrast, E-cadherin and Vimentin were reduced in MCF10A-Cx32 cells compared with controls over 7 days, and the expression pattern for nuclear Cx32 and Zeb2 was following similar pattern in MCF10A cells. Our results suggest a previously unknown time-dependent relation between Cx32 and the regulation of the EMT process.

Key words**:** *breast cancer; connexin32; epithelial-to-mesenchymal transition; nucleus; localization.*

ЗВ'ЯЗОК МІЖ КЛІТИННОЮ ЛОКАЛІЗАЦІЄЮ CX32 ТА ЕПІТЕЛІАЛЬНО-МЕЗЕНХІМАЛЬНИМ ПЕРЕХОДОМ У КЛІТИНАХ МОЛОЧНОЇ ЗАЛОЗИ

Конексини (Cx) відіграють у клітинах роль, яка і пов'язана з щілинними контактами, і незалежна від них, і їхня локалізація має важливе значення для їхньої функції в клітинних процесах. Окрім мембранної локалізації, конексини також можуть бути локалізовані в цитоплазмі та ядрі, особливо в ракових клітинах. На різних стадіях раку спостерігається диференційована локалізація конексинів, включно з Cx32. Cx32 був підвищений і спостерігався в цитоплазмі клітин у лімфатичних вузлах метастазів зразків раку молочної залози порівняно з первинними пухлинами. Однак, значення підвищення експресії Cx32 та зміни клітинної локалізації Cx32 при епітеліально-мезенхімальному переході (ЕМП) невідоме. Щоб визначити, чи протягом одного тижня надмірна експресія та/або локалізація Cx32 індукує процес ЕМП, ми спочатку дослідили клітинну локалізацію Cx32 у клітинах MCF10A та MDA-MB-231 у різні часові точки, використовуючи Вестерн-блот та RT-ПЛР, а також імунофарбування за допомогою конфокальної мікроскопії. Потім ми співвіднесли зміни експресії та локалізації Cx32 з експресією маркерів ЕМП. Ми показали, що Cx32 має змінену клітинну локалізацію, а гіперекспресія Cx32 підвищує рівень Slug, тоді як експресію E-кадгерину та Snail в MDA-MB-231 знижує впродовж 7 днів. На противагу цьому, в клітинах MCF10A-Cx32 експресія E-кадгерину та віментину знижувалась порівняно з контролем впродовж 7 днів, а експресія ядерного Cx32 та Zeb2 в клітинах MCF10A була подібною до контролю. Отримані результати свідчать про раніше невідомий зв'язок між Cx32 та регуляцією процесу ЕМП, який залежить від часу.

Ключові слова: рак молочної залози, конексин32, епітеліально-мезенхімальний перехід, ядро, локалізація.

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