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論　文　内　容　の　要　旨

氏　名　（　楊立立　）	
論文題名	Elucidation of the role of αB-crystallin in malignant breast cancer （難治性乳がんにおけるαB-crystallinの役割解明）

論文内容の要旨

Breast cancer is a complex and heterogeneous disease that causes 15%-20% of all deaths from cancer in women worldwide. Based on the expression of the human epidermal growth factor receptor 2 (HER2) and hormone receptor (HR), breast cancer can be divided into four subtypes. Among four subtypes, HER2-positive subtypes and triple negative breast cancer (TNBC) are known as aggressive subtypes of breast cancer. Although anti-HER2 (i.e., Trastuzumab) and chemotherapeutical applications have been used in clinical setting for HER2-positive and TNBC, partial of those HER2-positive and TNBC patients barely response to current and following therapy and experience rapid recurrence. The part of patients is recognized as malignant patients. Major reason of their recurrence is the aggressive tumorigenic and metastatic progress, however, the mechanism of therapy-induced aggressiveness of cancer cell has not been thoroughly understood. Hence, for the promotion of survival rate of breast cancer patients, it is an urgent necessity to investigate the molecular mechanism behind the therapy-induced aggressiveness of cancer cells.

Alpha-crystallin B chain (αB-crystallin) is a small heat-shock protein that plays crucial roles in multiple cellular processes. Recently, αB-crystallin is known to be associated with brain metastasis from clinical analyses of TNBC breast cancer patients. While its function in induction of aggressiveness of breast cancer cells is rarely known, the reports suggested the potential of αB-crystallin in aggressive feature of cancer cells. Therefore, this dissertation attempted to understand the role of αB-crystallin in therapy-induced aggressiveness of cancer cells.

In chapter one, by using proteomics, αB-crystallin was found to be upregulated in trastuzumab-resistant HER2-positive breast cancer cells (SKBR3-TR) in contrast to its parental sensitive cells (SKBR3). Besides, tube formation assay of endothelial cells, which is the one of the most widely used *in vitro* assay to model the reorganization stage of angiogenesis, showed that supernatant of SKBR3-TR significantly promoted the tube formation comparing to that of SKBR3. Moreover, it has been revealed that silencing αB-crystallin significantly repressed SKBR3-TR-induced tube formation. Mechanistically, mechanistic target of rapamycin (mTOR) in endothelial cells was activated by αB-crystallin. Besides, inhibition of mTOR reversed the promotion of tube formation which caused by SKBR3-TR. The αB-crystallin-activated mTOR in endothelial cells could also be suppressed by mTOR inhibitor. Collectively, this chapter revealed the upregulation of αB-crystallin in SKBR3-TR cells and clarified that supernatant from SKBR3-TR cells induced endothelial tube formation through the activation of mTOR in endothelial cells. These findings suggest that targeting the mTOR pathway through αB-crystallin might enhance trastuzumab efficiency. This knowledge could utilize to develop an inhibition method of αB-crystallin for the purpose of suppressing cell aggressiveness in trastuzumab resistance.

In chapter two, the role of αB-crystallin was observed in chemotherapy-induced motility of TNBC cells to verify our presumption that αB-crystallin might relate to the poor outcome of BL2 subtype of TNBC. First, αB-crystallin expression was analyzed in several cell line of TNBC subtypes. As a result, the expression level of αB-crystallin in BL2 subtype was greater than that in other subtypes. Therefore, the effect of chemotherapy on cell motility was evaluated by utilizing high-αB-crystallin expressed HCC1806 cells (BL2 subtype) and, as a comparison, low-αB-crystallin expressed MDA-MB-436 cells and MDA-

MB-231 cells (MSL subtype) to assess the malignant role of αB-crystallin in the differences of response to chemotherapy of TNBC. In the investigation of cell response to chemotherapy, the induction of cell motility by chemotherapy agents (i.e., fluorouracil and cisplatin) was evaluated in MDA-MB-231 cells and HCC1806 cells by using wound healing assay and transwell migration assay. The results showed that chemotherapy agent induced cell motility in high-αB-crystallin cells (HCC1806, MDA-MB-231 with forced expression of αB-crystallin) whereas suppressed that of low-αB-crystallin (MDA-MB-231, HCC1806 after silencing αB-crystallin). Collectively, it is illuminated that chemotherapy induced cell motility by αB-crystallin which might explain the poor outcome of BL2 subtype. These results enhance our understanding of chemotherapy-induced various drug response of TNBC subtypes. Moreover, these findings indicate that inhibitor of αB-crystallin would be developed to suppress cell motility and applied to improve survival rate of TNBC patients by combination with chemotherapy.

In this dissertation, the novel molecular function of αB-crystallin in malignant breast cancer is summarized in cellular level. Either in long-term treatment (i.e., trastuzumab resistance) or short-term treatment (i.e., 24-h treatment of 5-FU), αB-crystallin is involved in the aggressiveness of HER2-positve and TNBC cells that induced by drug therapy. Those results could enhance our knowledge on cancer cell evolution under drug selection and could be an important discovery in the field of medicine and pharmaceutical sciences, and contribute to further investigation of targeting αB-crystallin to improve the clinical outcome of breast cancer in the future.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究では、乳がんの中でも予後の悪い、HER2陽性乳がんとTNBCを対象に、乳がんの悪性化機序解明に向けて、(1) HER2（ハーツー）陽性乳がんにおける、トラスツズマブに対する獲得耐性ががん悪性化におよぼす影響とその分子機序の解明、(2) TNBC（トリプルネガティブ乳がん）における化学療法剤処置と転移能との連関解析などを多角的に試み、以下の学位論文に相応しい重要知見を得た。

1. HER2陽性細胞におけるトラスツズマブ獲得耐性において、αB-crystallinが管腔形成を促進することを見出した。

2. αB-crystallinがSKBR3-TR細胞の細胞遊走を亢進することを明らかとした

3. 5-FUとCDDPの処理による、TNBC細胞の細胞遊走の亢進に対して、αB-crystallinが促進的に働くことを見出した。

上記のように、これら知見は将来的に、乳がんの中でも予後の悪い、HER2陽性乳がんとTNBCを対象とした治療法開発の道を拓くものである。今後、さらに、HER2陽性乳がんとTNBCの悪性化機構における、αB-crystallinの役割解明を詳細に明らかとすることで、トラスツズマブ耐性乳がんおよびTNBCにおける悪性化機構の解明に貢献するものと期待される。

以上より、博士（薬科学）の学位論文に値するものと認める。