Overexpression of molecular chaperons GRP78 and GRP94 in CD44$^{hi}$/CD24$^{lo}$ breast cancer stem cells

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**Abstract**

**Introduction:** Breast cancer stem cell with CD44$^{hi}$/CD24$^{lo}$ phenotype is described having stem cell properties and represented as the main driving factor in breast cancer initiation, growth, metastasis and low response to anti-cancer agents. Glucose-regulated proteins (GRPs) are heat shock protein family chaperons that are charged with regulation of protein machinery and modulation of endoplasmic reticulum homeostasis whose important roles in stem cell development and invasion of various cancers have been demonstrated. Here, we investigated the expression levels of GRP78 and GRP94 in CD44$^{hi}$/CD24$^{lo}$ phenotype breast cancer stem cells (BCSCs).

**Methods:** MCF7, T-47D and MDA-MB-231 breast cancer cell lines were used. CD44$^{hi}$/CD24$^{lo}$ phenotype cell population were analyzed and sorted by fluorescence-activated cell sorting (FACS). Transcriptional and translational expression of GRP78 and GRP94 were investigated by western blotting and quantitative real time PCR.

**Results:** Results showed different proportion of CD44$^{hi}$/CD24$^{lo}$ phenotype cell population in their original bulk cells. The ranking of the cell lines in terms of CD44$^{hi}$/CD24$^{lo}$ phenotype cell population was as MCF7<T-47D<MDA-MB-231. Our results also indicated that CD44$^{hi}$/CD24$^{lo}$ phenotype cells exhibited higher mRNA and protein expression level of GRP78 and GRP94 compared to their original bulk cells.

**Conclusion:** Our results show a relationship between overexpression of GRP78 and GRP94 and exhibiting CD44$^{hi}$/CD24$^{lo}$ phenotype in breast cancer cells. We conclude that upregulation of GRPs may be an important factor in the emergence of CD44$^{hi}$/CD24$^{lo}$ phenotype BCSCs features.

**Keywords:** Breast cancer, Cancer stem cell, GRP78, GRP94, Overexpression

**Introduction**

Cancer stem cell theory propagates cancers arise by direction of minor subset of stem/progenitor cells inside tumors. Breast cancer stem cells (BCSCs) with CD44$^{hi}$/CD24$^{lo}$ phenotype inside breast tumors are known to have tumor-initiating behavior with stem cell-like characteristics, enhanced invasive properties and radiation resistance. CD44$^{hi}$/CD24$^{lo}$ phenotype cells were able to self-renewal, to maintain its subpopulation in tumor and to differentiate into downstream tumor cells resembling the composition of the original tumor. Evidences support prominent role of CD44$^{hi}$/CD24$^{lo}$ phenotype BCSCs in metastasis, resistant to chemotherapy and therefore responsible for cancer relapse. These cells exhibit a distinct gene expression signature that allows them to act as core engine for breast cancer malignancy. CD44$^{hi}$/CD24$^{lo}$ phenotype BCSCs possess many genetic and epigenetic features which are common in both cancerous and normal stem cells. It is due to significant molecular properties including altered response mechanism to DNA damage in BCSC that grant the cells to survival, invasion and chemoresistance. Essentially, rapid growth of tumor leads to undesirable metabolic environment such as, hypoxic and nutrient deprived, having reduced amounts of both amino acids and glucose. Nutrient and hypoxic stressors damage protein machinery and consequently cause unfolded/misfold-
ed protein accumulation in endoplasmic reticulum (ER) lumen. This phenomenon eventually activates unfolded protein response and ER stress.\textsuperscript{7,8} ER stress acts as regulator of cell homeostasis in response to aberrant synthesis machinery through controlling calcium balance and employment of ER chaperon proteins.\textsuperscript{9} Glucose Regulated Protein 78 and 94 kDa (GRP78 and GRP94) are heat shock protein family molecular chaperons that are found in the lumen of the ER. GRPs are essential regulator of ER function due to their function in protein translocation, folding and assembly, targeting malformed protein for degradation, ER Ca\textsuperscript{2+} binding and controlling the initiation of ER stress sensors.\textsuperscript{9,10} Expression of GRPs is also increases under growth conditions in particular glucose starvation. Studies showed that GRPs were essential for embryonic cell growth and its function is obligatory for early embryonic development.\textsuperscript{10-12} Altered GRPs expression has been reported in various cancers. GRPs are thought to play key roles in cancer cell survival, proliferation, invasion and several pathologic conditions such as poor prognosis and resistance to anticancer therapy.\textsuperscript{13-15} GRPs exhibit an anti-apoptotic function to block activity of apoptosis and autophagy, and eventually leading to death inhibition and increased cell survival.\textsuperscript{13-15} Emerging evidences suggest that GRPs may be exploited as negative factors for death inducing approaches such as radiotherapy as well as may be a diagnostic marker for breast cancer chemo-responsiveness.\textsuperscript{16} It has been shown that chemotheraphy against breast cancer leads to increasing level of GRPs in viable tumor tissues. Recent studies reported that cell surface GRP78 are required for regulation of hematopoietic, fetal and adult mammary stem cells quiescence that aid the stem cells to restore homeostasis.\textsuperscript{16,17} The reports also revealed pivotal function of GRP78 and GRP94 in mammmary tissue development.\textsuperscript{18,19} Interestingly, a recent report demonstrated that expression of GRP78 increases resistance of the breast cancer stem cell-like cells against radiotherapy.\textsuperscript{20} Moreover, we recently showed that chemical-induced ER stress in MCF7 cells suppresses CD44\textsuperscript{lo}/CD24\textsuperscript{hi} phenotype BCSCs subpopulation and eventually inhabits cell migration and invasion.\textsuperscript{21} Relying on these findings, we believe that ER stress and CD44 expression status of ER shaperons GRP78 and GRP94 in BCSCs. The reports also revealed pivotal function of GRP78 and GRP94 in mammmary tissue development.\textsuperscript{18,19} Interestingly, a recent report demonstrated that expression of GRP78 increases resistance of the breast cancer stem cell-like cells against radiotherapy.\textsuperscript{20} Moreover, we recently showed that chemical-induced ER stress in MCF7 cells suppresses CD44\textsuperscript{lo}/CD24\textsuperscript{hi} phenotype BCSCs subpopulation and eventually inhabits cell migration and invasion.\textsuperscript{21} Relying on these findings, we believe that ER stress and CD44 expression status of ER shaperons GRP78 and GRP94 in BCSCs.

### Materials and methods

#### Cell culture

MCF7, T-47D and MDA-MB-231 breast cancer cell lines were obtained from American Type Tissue Culture Collection (Manassas, USA). The cells were grown in Dulbecco’s modified eagle medium (DMEM)/F12 containing 10% fetal calf serum (FCS; both from Gibco, Rockford, USA), supplemented with 2 mmol/mL L-glutamine (Gibco, Rockford, USA) by incubation at 37°C in a 5% CO\textsubscript{2} humidified incubator. After the cell culture reached 80% confluence, the cells were trypsinized with 0.25% trypsin (Sigma-Aldrich, St. Louis, USA) and harvested.

#### Flow cytometry and fluorescence-activated cell sorting (FACS)

FITC-conjugated monoclonal anti-human CD44 IgG (#555478) and PE-conjugated monoclonal mouse anti-human CD24 IgG (#555428) antibodies and their respective isotype controls were used (all from BD Biosciences, San Diego, USA). All flow cytometry and FACS procedures were done as described previously.\textsuperscript{22}

#### Real time quantitative PCR

Total RNA from original cell lines and sorted CD44\textsuperscript{lo}/CD24\textsuperscript{hi} phenotype cells was extracted using TRIzol reagent (Invitrogen, Waltham, USA) according to protocol described previously.\textsuperscript{23} cDNA synthesis from total RNA was carried out using Transcripter High Fidelity cDNA Synthesis\textsuperscript{®} kit (Roche, Basel, Switzerland) by applying the oligo (dT)\textsubscript{15} primer pairs following the manufacturer’s instructions. Whole real time quantitative PCR reactions were done by employing an Applied Biosystems® 7500 real time PCR system and using TaqMan® Universal PCR Master Mix following the manufacturer’s instructions. Sequence of used primers and probes are shown in Table 1. Specific primers for PCR amplification were designed using IDT PrimerQuest software\textsuperscript{24} and synthesized by Biomers Inc. (Ulm, Germany). The cycle thresholds results were normalized to GAPDH as endogenous control. The expression levels were calculated by 2\textsuperscript{-ΔΔCt}.

#### Western blotting

Polyclonal rabbit anti-human GRP78 IgG (#G9043), Polyclonal goat anti-human GRP94 IgG (#G4545), rabbit anti-human GAPDH IgG (#G9545) and HRP conjugated goat anti rabbit IgG (#A0545) were purchased from Sigma-Al-

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**Table 1. Sequences of primers and TaqMan probes used for quantitative real time qPCR**

| Target cDNA (RefSeq) | Forward Primer | Reverse Primer | Probe Sequence |
|---------------------|----------------|----------------|----------------|
| GRP78 (NM_005347.4) | 5′-GGTGGGATCAAGGCTGATAGGAG-3′ | 5′-CTACACGCGCCAGCTATAA-3′ | /FAM/TGTGGGCAAGGTCATCCCTGAG/BHQ/-3′ |
| GRP94 (NM_003299.2) | 5′-GGCAGAGGACATCCTGACAA-3′ | 5′-CCTAATGCTGAGACAGAT-3′ | /FAM/TGCCAACATGGTGAAACTCCATCT/BHQ/-3′ |
| GAPDH (NM_002046.4) | 5′-TCCAGAAGATATCATCCTGGC-3′ | 5′-CTCTGCGGTTCGCTGAGG-3′ | /FAM/TGTGGGCAAGGTCATCCCTGAG/BHQ/-3′ |

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Fig. 2A

Overexpression of GRP78 and GRP94 in breast cancer stem cells was investigated. The results indicated that CD44+/CD24− phenotype BCSCs show a higher level of expression of GRP78 and GRP94 when compared with their original cell lines.

Discussion

In this study, we investigated GRP78 and GRP94 gene expression in the CD44+/CD24− phenotype cell subpopulations of MCF7, T-47D and MDA-MB-231 cell lines. CD44+/CD24− cells in breast cancers are represented possessing progenitor/stem cell properties and were known as core engine of tumor growth, invasion, and resistance to anti-cancer agents. Positive impact of BSCS population in generation of tumor in animal models, metastasis and response to anti-cancer therapies has been reported previously.1-6 In present study we showed different proportion of CD44+/CD24− phenotype cell subpopulation in MCF7, T-47D and MDA-MB-231 cell lines. The statistical significances were considered applying p<0.05 was considered as statistically significant.

Results

Overexpression of GRP78 and GRP94 in CD44+/CD24− phenotype BCSCs

Fig. 1

(a) Flow cytometry dot plate results of CD44+/CD24− phenotype cell subpopulation in breast cancer cell lines. Cells in Q4 correspond to CD44+/CD24− phenotype cells. An isotype antibodies corresponding to MDA-MB-231 cells was used as control. (B) Percentage of CD44+/CD24− phenotype cell subpopulation which was identified in breast cancer cell lines by.

* p < 0.05, ** p < 0.01.

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that holding unique genetic and epigenetic feature may be the main productive trend of BCSCs to exhibit stemness properties. Here, we showed higher levels of GRP78 and GRP94 expression in CD44<sup>hi</sup>/CD24<sup>lo</sup> phenotype BCSCs derived from MCF7, T-47D and MDA-MB-231 cell lines (Fig. 3). Significant function of GRPs in developmental processes and stem cell biology implicates that GRPs may be key factors for stem cell to manifest pluripotency behavior, although the exact mechanism remains unknown. Thus we speculate that overexpressed GRP78 and GRP94 in BCSCs may due to of the molecular regulators which push the cells to emerge progenitor/stem cell like behavior. In other words, higher level GRPs in the cells may arise from stem cell-like function in these cells. Regarding to a rational theory suggesting cancer stem cell origins from normal somatic stem cells, it is more probable to note higher levels of GRP78 and GRP94 from normal somatic stem cells. GRPs are essential for ER homeostasis and cell survival under ER stress. GRPs evacuate malfolded proteins in ER lumen and their assistance in modulation of homeostasis made it as advantageous factor for tumor survival and resistance in stressful conditions. GRP78 was found as an overexpressed gene during breast cancer invasion specially metastasis to lymph nodes. A recent investigation illustrated a novel GRP78 function in deactivation of apoptotic paths in breast cancer. According to this report, GRP78 inhibits BIK protein binding to BCL-2 in breast cancer cells. This suggests that GRP78 may be a responsible factor in endocrine resistance in breast cancer. Higher level of GRP94 expression was also observed in breast carcinoma in comparison with normal tissue. Moreover, investigating GRP94 expression in HER2 overexpressed breast cancers, revealed that inhibition of GRP94 could destabilizes HER2 and inhibited RAF1–MAPK survival signaling at tumor cell membrane. HER2 leads to activation of different downstream signaling cascades, including the MAPK, a key pathway for proliferation, and also a set of critical factors which may lead to increased cell proliferation, motility, decreased apoptosis, angiogenesis and resistance against therapy. Silencing of GRP94 causes inhibition of proliferation and migration in MDA-MB-231 breast cancer cell line. These data may support the notion that overexpression of GRPs may be a hallmark for breast malignancy that it has been intertwined with breast tumor molecular abnormalities. Thus, it may think to be a part of breast cancer pathophysiology. In addition, it can exclude ER stress-mediated apoptosis and autophagy in vitro and in vivo tumor models. Many studies demonstrated ER stress role in suppression of cancers. In a recent study, we demonstrated that CD44<sup>hi</sup>/CD24<sup>lo</sup> phenotype BCSCs are vulnerable against ER stress. Induced ER stress in breast cancer bulk cells inhibits cell proliferation and invasion via promoting cell death in parallel with suppressed population of CD44<sup>hi</sup>/CD24<sup>lo</sup> phenotype cells. In the other hand, autophagy is a con-
sequence phenomenon of ER stress.\textsuperscript{31} In another report, we showed that CD44\textsuperscript{hi}/CD24\textsuperscript{lo} phenotype cancer stem cell subpopulation declines under autophagic condition.\textsuperscript{32} GRPs play crucial negatively regulatory roles in ER stress. GRP78 and GRP94 knockout mice models showed that deletion of these genes led to a dramatic reduction in tumor angiogenesis and metastatic growth and increasing apoptosis in tumor tissue.\textsuperscript{27,32,33} A recent interesting report illustrated that GRP78 knockout CD44\textsuperscript{lo}/CD24\textsuperscript{hi} phenotype cells showed very lower tumorigenesis, compared with GRP78 wild-type CD44\textsuperscript{lo}/CD24\textsuperscript{hi} phenotype cells. Silencing GRP78 in CD44\textsuperscript{hi}/CD24\textsuperscript{lo} phenotype cells increased chemo-radiosensitivity and inhibited cell invasion and reverse epithelial-mesenchymal transition.\textsuperscript{34} This may relate that GRP78 has important functions in CD44\textsuperscript{hi}/CD24\textsuperscript{lo} phenotype cells like other phenotype tumor cells. In summary, GRPs play key role in normal breast tissue, adult stem cells and also breast tumor cells survival and development. Therefore, we suggest that expression status of GRPs is the common aspect between distinct phenotype cells in breast tumor. It is important to investigate the linkage of GRPs and breast cancer stem cells properties including self-renewal, differentiation and resistance. Relying on these findings, we suppose that overexpression of GRP78 and GRP94 in the BCSCs may be part of the intrinsic biology of these types of cancer cells due to its function in exhibition of both tumor and stem cell characteristics, however the reason of up-regulation is not clear yet. There are not significant reports concerning expression profile of breast cancer stem cells yet. This study is the first report implicating overexpression of GRPs in breast cancer stem cells. In many reports, GRPs have been known as an oncogene which is suggested to be a strong candidate targets in breast cancer therapy. Thus, we strongly encourage future investigations to clarify potential of GRPs to be used as target for cancer therapy.

Conclusion
This report shows that different breast cancer cell lines exhibit dissimilar contents of CD44\textsuperscript{hi}/CD24\textsuperscript{lo} phenotype cells. Our findings suggest overexpression of GRP78 and GRP94 genes in CD44\textsuperscript{lo}/CD24\textsuperscript{hi} phenotype BCSCs in comparison with the original cell lines suggesting a relationship between expression of GRPs and exhibition of CD44\textsuperscript{lo}/CD24\textsuperscript{hi} phenotype in the cell lines (Fig. 3). Given that GRPs share similar signature in adult stem cells, breast tissue and breast tumor cells gene expression profile, we conclude that GRPs could play an important role in exhibition cancer stem cell properties and overexpression may be a hallmark for CD44\textsuperscript{lo}/CD24\textsuperscript{hi} phenotype BCSCs.

Ethical approval
Not applicable.

Competing interests
Authors declare no conflict of interests.

References
1. Kreso A, Dick JE. Evolution of the cancer stem cell model. Cell Stem Cell. 2014; 14: 275–91. doi:10.1016/j. stem.2014.02.006
2. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 2003; 100: 3983–8. doi:10.1073/pnas.0530291100
3. Fillmore CM, Kuperwasser C. Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy. Breast Cancer Res. 2008; 10: R25. doi:10.1186/bcr1982
4. Vinogradov S, Wei X. Cancer stem cells and drug resistance: the potential of nanomedicine. Nanomedicine (Lond). 2012; 7: 597–615. doi:10.2217/nmm.12.22
5. Lin X, Li J, Yin G, Zhao Q, Elias D, Lykkesfeldt AE, et al. Integrative analyses of gene expression and DNA methylation profiles in breast cancer cell line models of tamoxifen-resistance indicate a potential role of cells with stem-like properties. Breast Cancer Res. 2013; 15: R119. doi:10.1186/bcr3588
6. Abdullah LN, Chow EK-H. Mechanisms of chemoresistance in cancer stem cells. Clin Transl Med. 2013; 2: 3. doi:10.1186/2001-1326-2-3
7. Scheuner D, Song B, McEwen E, Liu C, Laybutt R, Gillespie P, et al. Translational control is required for the unfolded protein response and in vivo glucose homeostasis. Mol Cell. 2001; 7: 1165–76.
8. Hammad M, Oulidi A, Gackiere F, Katsogiannou M, Slomianny C, Roudbaraki M, et al. Modulation of ER stress and apoptosis by endoplasmic reticulum calcium leak via translocon during unfolded protein response: involvement of GRP78. FASEB J Off Publ Fed Am Soc Exp Biol. 2013; 27: 1600–9. doi:10.1096/ fj.12-218875
9. Luo B, Lee AS. The critical roles of endoplasmic reticulum chaperones and unfolded protein response in tumorigenesis and anticancer therapies. Oncogene.
Grp78 mediates mammary gland development. Zhu G, Wang M, Spike B, Gray PC, Shen J, Lee S-H, et al. J Biol Chem. 2014; 289: 25687–96. doi:10.1074/jbc.M607007200

Li B, Cheng XL, Yang YP, Li ZQ. GRP78 mediates mammary gland development. J Biol Chem. 2014; 289: 25687–96. doi:10.1074/jbc.M607007200

Li B, Tseng C-C, Adams GB, Lee AS. Deficiency of Grp78 in the hematopoietic system alters proliferation regulators in hematopoietic stem cells. Stem Cells Dev. 2013; 22: 3062–73. doi:10.1089/scd.2013.0181

Spoke BT, Kelber JA, Booker E, Kalathur M, Rodewald R, Lipianskaja J, et al. CRIP/GRP78 signaling maintains fetal and adult mammary stem cells ex vivo. Stem cell reports. 2014; 2: 427–39. doi:10.1016/j.stemcr.2014.02.010

Zhu G, Wang M, Spike B, Gray PC, Shen J, Lee S-H, et al. Differential requirement of GRP94 and GRP78 in mammary gland development. Sci Rep. 2014; 4: 5390. doi:10.1038/srep05390

Li B, Cheng XL, Yang YP, Li ZQ. GRP78 mediates radiation resistance of a stem cell-like subpopulation within the MCF7 breast cancer cell line. Oncol Rep. 2013; 30: 2119–26. doi:10.3892/or.2013.2710

Nami B, Donmez H, Kocak N. Tunicamycin-induced endoplasmic reticulum stress reduces in vitro subpopulation and invasion of CD44+/CD24- phenotype breast cancer stem cells. Exp Toxicol Pathol. 2016; 4–11. doi:10.1016/j.etp.2016.06.004

Nami B, Donmez H, Kocak N. Autophagy reduces subpopulation of CD44+ / CD24− phenotype cancer stem cells in MCF7 and Hep-2 cells culture. J Cancer Stem Cell Res. 2015; 3: 1. doi:10.14343/JCSR.2015.3e1002

Rio DC, Ares MJ, Hannon GJ, Nilsen TW. Polyacrylamide gel electrophoresis of RNA. Cold Spring Harb Protoc. 2010; 2010: pdb.prot5444. doi:10.1101/pdb.prot5444

IDT. PrimerQuest® program. Coralville, USA. http://www.idtdna.com/Scitools. [Internet]. 2012.

Holliday DL, Speirs V. Choosing the right cell line for breast cancer research. Breast Cancer Res. 2011; 13: 215. doi:10.1186/bcr2889

Bhat-Nakshatri P, Appaiha H, Ballas C, Pick-Franke P, Goulet RJ, Badve S, et al. SLUG/SNAI2 and tumor necrosis factor generate breast cells with CD44+/CD24− phenotype. BMC Cancer. 2010; 10: 411. doi:10.1186/1471-2407-10-411

Dejeans N, Glorieux C, Guenin S, Beck R, Sid B, Rousseau R, et al. Overexpression of GRP94 in breast cancer cells resistant to oxidative stress promotes high levels of cancer cell proliferation and migration: implications for tumor recurrence. Free Radic Biol Med. 2012; 52: 993–1002. doi:10.1016/j.freeradbiomed.2011.12.019

Patel PD, Yan P, Seidler PM, Patel HJ, Sun W, Yang C, et al. Paralog-selective Hsp90 inhibitors define tumor-specific regulation of HER2. Nat Chem Biol. 2013; 9: 677–84. doi:10.1038/nchembio.1335

Pyuko P, Kardosh A, Liu Y-T, Soriano N, Xiong W, Chow RH, et al. Calcium-activated endoplasmic reticulum stress as a major component of tumor cell death induced by 2,5-dimethyl-celecoxib, a non-coxib analogue of celecoxib. Mol Cancer Ther. 2006; 5: 1537–46. doi:10.1158/1535-7163.MCT-06-0629

Schonthal AH. Pharmacological targeting of endoplasmic reticulum stress signaling in cancer. Biochem Pharmacol. 2013; 85: 653–66. doi:10.1016/j.bcp.2012.09.012

Yorimitsu T, Nair U, Yang Z, Kliouisky D. Endoplasmic reticulum stress triggers autophagy. J Biol Chem. 2006; 281: 30299–304. doi:10.1074/jbc.M607007200

Vandewynckel Y-P, Laukens D, Geerts A, Bogaerts E, Paridaens A, Verhelst X, et al. The paradox of the unfolded protein response element and GATA-4. Growth Factors. 2009; 27: 37–47. doi:10.1080/09230880903388932

Pan Z, Erkan M, Streit S, Friess H, Kleeff J. Silencing of GRP94 expression promotes apoptosis in pancreatic cancer cells. Int J Oncol. 2009; 35: 823–8. doi:10.3892/ijot.2013.1586

Chiu CC, Lee LY, Li YC, Chen YJ, Lu YC, Li YL, et al. Grp78 as a therapeutic target for refractory head-neck cancer with CD24−/CD44+ stemness phenotype. Cancer Gene Ther. 2013; 20: 606–15. doi:10.1038/cgt.2013.64