The role of CD24+ population in non-cancer stem cells

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In the current issue of the *Journal of the Chinese Medical Association*, Boo et al[1] demonstrated the phenotype and microRNA (miRNA) profiling of CD24 positive (CD24+) cell population in Michigan Cancer Foundation-7 (MCF7) spheroid. The CD24+ MCF7 cells were able to form secondary sphere as well as suppressed wound healing and invasion. Remarkably, the CD24+ MCF7 cells expressed high level of aldehyde dehydrogenase 1 (ALDH1), which considered as potential cancer stem cell (CSC) marker and involved in drug resistance.[1]

CSCs, also known as tumor-initiating cells, are a subpopulation of tumor cells which have capacities of self-renewal and differentiation. Rising evidence indicates that CSCs contribute to tumor initiation, growth, metastasis, and relapse. CSC surface markers render the targeted therapies for cancer treatment. $CD44^+CD24^-$ and ALDH1 are widely used markers of breast CSC.[2] Several studies deciphered the behaviors of CD44/CD24 subpopulation in tumorigenesis. Previous reports have shown that $CD44^+CD24^-$ sorted from parental MCF7 cells harbored highest invasive capacity and tumorigenicity. Both the $CD44^+CD24^-$ and $CD44^+CD24^+$ cells were able to form tumors, contrast to that $CD44^-CD24^-$ and $CD44^-CD24^+$ cells were not.[3] Interestingly, the signal transducer CD24, a small glycosylphosphatidylinositol-linked membrane glycoprotein, has been reported to be overexpressed in breast cancer tissue and has been implicated as a prognostic marker in breast cancer.[4] To date, the feature of CD24+ cell in spheroid is still ambiguous.

The present study showed that a small population of CD24+ cells from MCF7 spheroid was sorted. The CD24+ MCF7 cells were resistant against doxorubicin and cisplatin. Additionally, these CD24+ cells formed secondary spheroid with low levels of CSC markers including CD44, CD49f, Nanog, Oct4, and Sox2, but not ALDH1. It has been manifested the association between CD24 and drug resistance. Taxane induced a transient $CD44^{high}CD24^{high}$ state from non-CSC population in breast cancer cells. This transient state was resulted from engagement of CD44 and CD24 in lipid rafts. These cells exhibited chemotherapy resistance phenotype through Src family kinase (SFK) activation.[5] In hepatocellular carcinoma, CD24 overexpression resulted in autophagy-mediated sorafenib resistance through enhancing protein phosphatase 2A expression and inactivating Akt/mammalian target of rapamycin (Akt/mTOR) pathway.[6] On the other hand, ALDH1 expression was linked to aggressive phenotype and chemoresistance of breast cancer.[7] It has been reported that blockade of ALDH sensitized CSC to chemotherapy.[8] Furthermore, the roles of CD24 in CSC characteristic and tumorigenesis may dependent on types of cell and cancer as well as microenvironment. The present study showed that CD24+ cells sorted from MCF7 spheroid have epithelial-like features with less CSC phenotypes. However, $CD44^+CD24^+$ cells derived from xenograft exhibited mesenchymal phenotype, stemness properties, and proinflammatory signature while losing tumorigenicity. Intriguingly, the $CD44^+CD24^+$ cells expressed high level of ATP-binding cassette sub-family B member 1 gene, which encoding a member of ATP-binding cassette transporter and was responsible for multidrug resistance.[9] This evidence suggest that the transient CD24+ state is not responsible for tumorigenicity but involves in chemoresistance.

MiRNAs play a crucial role in cellular and biologic processes. Dysregulation of miRNAs is linked to various human malignancies. In the present study, the differential expression analysis was conducted to identify miRNAs that were relevant to CD24 in non-CSC cell subpopulation. MicroRNA (miR)-204, the most upregulated miRNA in CD24+ MCF7 cells, has been reported as tumor suppressor in breast cancer. Ectopic expression of miR-204 suppressed cell viability, migration, and tumor progression.[10] Some of the downregulated miRNAs in CD24+ MCF7 cells have been reported sensitizing breast cancer cells to doxorubicin, such as miR-30c, miR-181a, and miR-195.[11] Based on Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathway analysis, these regulated miRNAs in CD24+ MCF7 cells were involved in downregulation of CSC-related pathway and upregulation in adherens junction pathway. The downregulated KEGG pathways including Wnt and Hedgehog pathways were implicated in breast CSC self-renewal. Inhibition of Wnt/β-catenin signaling suppressed breast CSC self-renewal. Let-7 and microRNA-600 have been reported that modulating CSC maintenance through Wnt pathway. The Hedgehog ligand sonic hedgehog enhanced breast CSC self-renewal and multilineage differentiation.[12] However, the additional experiments are needed to validate these miRNAs and KEGG pathways mediating CD24+ phenotypes.

Sphere-formation assay is commonly used to enrich CSC subpopulation *in vitro*. Previous studies indicated heterogeneity of spheres among breast cancer cell lines and primary human mammary epithelia. The CSC subpopulations sorted by fluorescence-activated cell sorting were not homogenous enriched in spheres. Diversity of morphologies and phenotypes in spheres of basal-like and luminal-like breast cancer cell lines has been

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observed.[13] In addition, identifying the breast CSC using different markers, such as $CD44^+CD24^-$, CD133, and ESA^+, exhibited different biologic and pathologic phenotypes.[14] Moreover, more research is needed to characterize the $CD24^+$ population in organoid cultures of primary human breast cancers or in patient-derived xenografts of breast cancers, which would be the most representative of the tissue origin. Nevertheless, the present study provides the CD24-related miRNAs and pathways regarding CSC and drug resistance in non-CSC population.

REFERENCES

1. Boo L, Yeap SK, Ali NM, Ho WY, Ky H, Satharasinghe DA, et al. Phenotypic and microRNA characterization of the neglected CD24+ cell population in MCF-7 breast cancer 3-dimensional spheroid culture. *J Chin Med Assoc* 2020;**83**:67–76.
2. Sun HR, Wang S, Yan SC, Zhang Y, Nelson PJ, Jia HL, et al. Therapeutic strategies targeting cancer stem cells and their microenvironment. *Front Oncol* 2019;**9**:1104.
3. Yan W, Chen Y, Yao Y, Zhang H, Wang T. Increased invasion and tumorigenicity capacity of CD44+/CD24- breast cancer MCF7 cells in vitro and in nude mice. *Cancer Cell Int* 2013;**13**:62.
4. Jing X, Cui X, Liang H, Hao C, Yang Z, Li X, et al. CD24 is a potential biomarker for prognosis in human breast carcinoma. *Cell Physiol Biochem* 2018;**48**:111–9.
5. Goldman A, Majumder B, Dhawan A, Ravi S, Goldman D, Kohandel M, et al. Temporally sequenced anticancer drugs overcome adaptive resistance by targeting a vulnerable chemotherapy-induced phenotypic transition. *Nat Commun* 2015;**6**:6139.
6. Lu S, Yao Y, Xu G, Zhou C, Zhang Y, Sun J, et al. CD24 regulates sorafenib resistance via activating autophagy in hepatocellular carcinoma. *Cell Death Dis* 2018;**9**:646.
7. Kida K, Ishikawa T, Yamada A, Shimada K, Narui K, Sugae S, et al. Effect of ALDH1 on prognosis and chemoresistance by breast cancer subtype. *Breast Cancer Res Treat* 2016;**156**:261–9.
8. Januchowski R, Wojtowicz K, Zabel M. The role of aldehyde dehydrogenase (ALDH) in cancer drug resistance. *Biomed Pharmacother* 2013;**67**:669–80.
9. Ortiz-Montero P, Liu-Bordes WY, Londoño-Vallejo A, Vernot JP. CD24 expression and stem-associated features define tumor cell heterogeneity and tumorigenic capacities in a model of carcinogenesis. *Cancer Manag Res* 2018;**10**:5767–84.
10. Hong BS, Ryu HS, Kim N, Kim J, Lee E, Moon H, et al. Tumor suppressor miRNA-204-5p regulates growth, metastasis, and immune microenvironment remodeling in breast cancer. *Cancer Res* 2019;**79**:1520–34.
11. Si W, Shen J, Zheng H, Fan W. The role and mechanisms of action of microRNAs in cancer drug resistance. *Clin Epigenetics* 2019;**11**:25.
12. Nalla LV, Kalia K, Khairnar A. Self-renewal signaling pathways in breast cancer stem cells. *Int J Biochem Cell Biol* 2019;**107**:140–53.
13. Smart CE, Morrison BJ, Saunus JM, Vargas AC, Keith P, Reid L, et al. In vitro analysis of breast cancer cell line tumourspheres and primary human breast epithelia mammospheres demonstrates inter- and intrasphere heterogeneity. *PLoS One* 2013;**8**:e64388.
14. Hwang-Verslues WW, Kuo WH, Chang PH, Pan CC, Wang HH, Tsai ST, et al. Multiple lineages of human breast cancer stem/progenitor cells identified by profiling with stem cell markers. *PLoS One* 2009;**4**:e8377.