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ΔNp63α induces quiescence and downregulates the BRCA1 pathway in estrogen receptor-positive luminal breast cancer cell line MCF7 but not in other breast cancer cell lines

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ABSTRACT

Despite apparent resection of tumors, breast cancer patients often suffer relapse due to remnant dormant tumor cells. Although quiescence of cancer stem cells is thought as one of the mechanisms regulating dormancy, the mechanism underlying quiescence is unclear. Since ΔNp63α, an isoform of p51/p63, is crucial in the maintenance of stem cells within mammary epithelium, we investigated its roles in the regulation of dormancy in normal and malignant breast cells. Inducible expression of ΔNp63α in MCF7 estrogen receptor positive (ER+) luminal breast cancer cells led to quiescence and acquisition of progenitor-like properties. Judging from mRNA-microRNA microarray analysis, activation of bone morphogenetic protein (BMP) signaling and inhibition of Wnt signaling emerged as prominent mechanisms underlying ΔNp63α-dependent induction of quiescence and acquisition of stemness in MCF7. More interestingly, through Ingenuity Pathway analysis, we found for the first time that BRCA1 pathway was the most significantly downregulated pathway by ΔNp63α expression in quiescent MCF7 cells, where miR-205 was a downstream mediator. Furthermore, ΔNp63α-expressing MCF7 cells exhibited resistance to paclitaxel and doxorubicin. Expression of ΔNp63α in normal MCF10A basal cells increased proliferation and stemness, but did not affect more aggressive luminal (T47D) and basal (MDA-MB-231) cells with p53 mutation. Gene expression datasets analyses suggested that ΔNp63 expression is associated with relapse-free survival of luminal A/B-type patients, but not of the other subtypes. Our results established a cell type-specific function of ΔNp63α in

Abbreviations: ER, estrogen receptor; BMP, bone morphogenetic protein; DTC, disseminated tumor cell; CTC, circulating tumor cell; TNBC, triple negative breast cancer; BLBC, basal-like breast cancer; BCSC, breast cancer stem cell; GO, gene ontology; IPA, ingenuity pathway analysis; Dox, doxycycline.

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induction of quiescence and downregulation of the BRCA1 pathway which suggested a role of ΔNp63α in the dormancy of luminal breast cancers.

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1. Introduction

Many breast cancer patients suffer relapse years after removal of the primary tumor. Dormancy in disseminated tumor cells (DTCs) presumably critically contributes to relapse (Aguirre-Ghiso, 2007). Quiescence, a reversible, non-dividing state of adult stem cells, is thought to contribute to tumor dormancy and poses therapeutic challenges since conventional therapies mainly target proliferating cells (Aguirre-Ghiso, 2007; Cheung and Rando, 2013). Although the mechanisms of cellular quiescence have been studied in different physiological systems (Li and Clevers, 2010), the mechanisms regulating quiescence in breast cancer cells remain unclear.

Mammary tissue undergoes several developmental stages including embryonic, pubertal, pregnancy, lactation, and post-lactation stages and undergoes repetitive cycles of maturation and involution in each pregnancy (Hennighausen and Robinson, 2001). Therefore, it inevitably contains multiple types of stem and progenitor cells, which undergo cycles of quiescence and proliferation upon appropriate hormonal signals including estrogen, progesterone and prolactin. Hence, mammary tissue is susceptible to carcinogenesis.

Breast cancer is a heterogeneous disease encompassing several molecular subtypes with specific disease progression mechanisms. Based on gene expression profiling, breast cancers are classified into molecular subtypes including luminal A/B, basal-like, epidermal growth factor receptor 2 (ERBB2/HER2)-overexpressing, and normal or claudin-low (Perou et al., 2000). Nonetheless, this exhaustive expression analysis failed to separate the clinically important class triple-negative breast cancer (TNBC) lacking estrogen receptor (ER), progesterone receptor (PR), and HER2. Furthermore, additional TNBC subtypes including basal-like (BL1 and BL2), immune modulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR) subtypes have been described, suggesting a heterogeneous nature of TNBCs (Chen et al., 2012; Chiorean et al., 2013; Jezequel et al., 2015; Lehmann et al., 2011). In addition, TNBCs and basal-like breast cancer (BLBC) significantly overlap (Rody et al., 2011) and are the most aggressive types, prone to relapse, metastasis, and early death. Furthermore, the *BRCA1/2* genes have been identified as genes predisposing women for breast and ovarian cancers (Welcsh and King, 2001). Germline mutation or loss of BRCA1, which is a crucial regulator of DNA repair pathways, is associated with highly aggressive basal-like breast cancer (Deng and Wang, 2003). Adding more complexity to the classification is the identification of breast cancer stem cells (BCSCs). These cells, which can be a population distinct from tissue stem cells, are critical for cancer management because they are capable of self-renewal and contribute to therapeutic resistance, recurrence, and metastasis (Al-Hajj et al., 2003; Kreso and Dick, 2014). Two distinct types of BCSCs have been proposed; one consists of epithelial-like BCSCs, which are proliferative and are thought to be localized in primary tumor or macrometastases, and the other are mesenchymal-like BCSCs, which are quiescent and probably occur in circulating tumor cells (CTCs) and bone marrow micrometastases (Liu et al., 2014). However, the existence of these distinct types of BCSCs is still debated and further verification is necessary. Recently, breast cancers have additionally been categorized according to the differentiation status of mammary cells, which tumor cells originate from. In normal mammary epithelial differentiation, stem cells differentiate into bipotential progenitors, which undergo further differentiation into basal/myoepithelial and luminal progenitor cells. Luminal progenitors have been identified as the origin of basal-like and HER2+ breast cancer, whereas claudin-low tumors originate from mammary stem cells (Prat and Perou, 2009).

Recent evidence suggests that quiescent stem cells ($PKH26^{+}$) in normal mammary gland and breast cancer are positive for p63, a p53 tumor suppressor family member (Pece et al., 2010). p63 (initially named “p51” by us) plays an unprecedented role in various developmental processes and cancer (Osada et al., 1998; Yang et al., 1998). ΔNp63, an N-terminally truncated isoform, is predominantly expressed in the basal/myoepithelial layer of the mammary gland (Nylander et al., 2002). It has been reported to be involved in regulating mammary stem cell quiescence (Li et al., 2008), mammary epithelial integrity (Carroll et al., 2006), lactation (Forster et al., 2014), BLBC prevention (Buckley et al., 2011), and metastasis suppression (Bergholz et al., 2014). Furthermore, ΔNp63 is shown as a potential BLBC marker (Ribeiro-Silva et al., 2005) and prosurvival factor in HER2 tumorigenesis (Yallowitz et al., 2014). Although p63 function has been extensively studied, the role of ΔNp63α in luminal ER+ breast cancers, in which tumor dormancy is prevalent, is unclear (Zhang et al., 2013). Elucidation of the precise functions of p63 in each type of breast cancer cell line, especially in luminal ER+ breast cancer cells, may give valuable information on breast cancers given the heterogeneity of their origin.

Here, we studied the role of p63 in luminal, ER+ breast cancer cells; we inducibly expressed ΔNp63α in MCF7 luminal, ER+ breast cancer cells to study its roles in proliferation, stemness, and therapeutic response. We used various other cell lines to elucidate p63 function in distinct cell types and to obtain additional information on different breast cancer subtypes. In addition, we analyzed ΔNp63 expression in public gene expression datasets and correlated our findings with the clinical outcomes of patients with different subtypes of breast cancer.

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