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Review

# $Na^+/H^+$ exchanger-mediated hydrogen ion extrusion as a carcinogenic signal in triple-negative breast cancer etiopathogenesis and prospects for its inhibition in therapeutics

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ABSTRACT

Breast cancer is the leading cause of cancer-related death in women in Europe and North America, and metastasis is the primary cause of fatality in patients with breast cancer. While some breast cancers are quite treatable, the triple-negative breast cancers are more metastatic and resistant to chemotherapy. There is clearly an urgent need for better treatments for this form of the disease. Breast cancer is characterized by genetically complex intra-tumour heterogeneity, particularly within the triple-negative clinical subtype. This complicates treatment options, so the development of specifically targeted chemotherapy for less treatable forms is critical. Dysregulation of pH homeostasis is a common factor in breast tumour cells. This occurs in concert with a metabolic switch to aerobic glycolysis that occurs at the onset of oncogenic transformation. The $Na^+/H^+$ exchanger isoform 1 (NHE1) is the major pH regulatory protein involved in the increased proton extrusion of breast cancer cells. Its increased activity results in intracellular alkalinisation and extracellular acidification that drives cancer progression. The acidification of the extracellular tumour microenvironment also contributes to the development of chemotherapy resistance. In this review, we outline the role of $H^+$ as a carcinogenic signal and the role and regulation of NHE1 as a trigger for metastasis. We review recent evidence supporting the use of pharmacological inhibitors of NHE1 as a viable treatment option for triple-negative breast cancer.



**Contents**



## 1. Introduction

Breast cancer is the most commonly diagnosed cancer among women [1] and is the leading cause of cancer-related death in women in Europe and North America [2,3]. It will affect approximately 1 in 9 women in their lifetime. In breast cancer, it is the resulting metastasis that is the primary cause of fatality [4,5], with about 50% of all patients showing evidence of metastasis at first presentation [6]. Not all breast cancers are alike. Some are quite treatable and some are much less so. Triple-negative (TN) breast cancers (negative for estrogen, progesterone, and HER2 (ERBB2) receptors) are the most problematic, tending to be more metastatic and resistant to chemotherapy. While triple-negative breast cancer represents only 10–20% of breast cancers [7], this is still a large number of women. Only a minority of women with metastatic

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triple-negative breast cancer survive past 5 years, even with aggressive chemotherapy [8–10]. Despite advances in early detection and innovations in precision surgery aimed to conserve normal breast tissue, treatment strategies for patients with invasive breast cancer usually involve radiation and/or chemotherapy to curb the potential spread of malignant cells to sites beyond the breast. However, in both broad range and targeted chemotherapy regimens, the inherent heterogeneity of breast tumours presents a major challenge to treatment, so much so that breast cancer is categorized as multiple diseases based on subtype differentiation as opposed to one single disease [11]. The development of targeted chemotherapies where the complication of intra-tumour heterogeneity can be negated as an impediment to successful treatment is therefore an attractive and worthwhile prospect. Tumours are complex entities that include genetically and metabolically aberrant cancer cells, genetically stable stromal cells, and infiltrating immune cells. They are functionally dependent on the acidic, hypoxic, and poorly perfused serum-deprived microenvironment that surrounds them for cancer progression to occur [12,13]. Pharmacological targeting of this tumour microenvironment therefore represents an avenue for a putative widespread treatment strategy heretofore unexplored in great depth. pH homeostasis in cancer cells and, consequently, in the tumour microenvironment, is regulated by multiple pH regulatory proteins, chief of which is the $Na^+/H^+$ exchanger NHE1. NHE1 becomes activated by intracellular acidification in normal cells [14] but this process becomes dysregulated during oncogenic transformation [15]. In cancer cells, elevated NHE1 activity results in increased $H^+$ extrusion that leads to cellular alkalinisation and the establishment of the acidic extracellular tumour microenvironment [13]. Over the past decade, experts in the field of pH regulation and homeostasis in tumour cells have realized the potential of targeting the tumour microenvironment pH as an anti-cancer therapy [16–19]. In this review, we present evidence to highlight the role of $H^+$ ions as a key carcinogenic signal, regulating both intracellular pH of tumour cells and the extracellular pH of the tumour microenvironment. Several NHE1 inhibitors that curb $H^+$ extrusion and $Na^+$ uptake, and consequently re-establish acid-base balance in the tumour microenvironment, have been assessed for their efficacy in treatment in cancer models. We summarize recent progress and discuss options for tumour-targeted drug delivery.

## 2. Breast tumour heterogeneity: a hindrance to targeted therapy

Breast cancer is widely characterized as being of three major subtypes based on the expression of estrogen and/or progesterone receptors (ER+, PR+), an amplification of human epidermal growth factor receptor-2 (HER2++) expression, or the absence of all of these receptors (triple-negative breast cancer, TNBC) [20]. This taxonomy is based on histotyping; further classification by gene expression profiling reveals five intrinsic clinical subtypes: luminal A, luminal B, HER2-amplification, basal-like, and normal-like [21,22]. The luminal subtypes are hormone receptor-positive, while the basal-like subtype primarily exhibits a triple-negative profile [20]; the normal-like subtype is now known to have been identified from a mixed sample of breast carcinoma and normal breast cells [22,23]. Determination of treatment strategies depends on these characterizations: endocrine therapy targeted to hormone receptor-positive subtypes, and anti-HER2 targeted therapy (*e.g.* trastuzumab) for the HER2++ subtype; these targeted therapies generally translate into improved treatment outcomes. Treating triple-negative basal-like breast cancer is complicated by the inability to target hormone or HER2 receptors. Nonspecific cytotoxic chemotherapy therefore remains the cornerstone of treatment strategies for these patients and, while outcomes can initially be favourable, overall prognoses and patient survival rates are poor [23–25], and the development of chemotherapy resistance is problematic [11].

Amongst invasive breast cancers, the incidence of triple-negative breast cancer (TNBC) ranges from 15 to 20% [25]. TNBC is the most clinically aggressive of all the subtypes, with high recurrence rates in the early years post-treatment, and an increased tendency towards distant metastasis, higher grades, and large tumours with a greater infiltration of lymphocytes [23,26]. TNBC also occurs at higher frequencies in younger patients, those carrying the BRCA1 mutation, and those of African-American and Hispanic origin [23,27,28]. In recent years, gene expression profiling revealed further complications to therapy options by identifying six distinct subtypes of TNBC tumours: *1,* a highly proliferative basal-like subtype with an increased expression of cell cycle and DNA damage response genes (BL1); *2,* a second basal-like subtype with elevated expression of growth factor receptors and myoepithelial markers (BL2); *3,* an immunomodulatory (IM) subtype typified by an upregulation in genes involved in immune processes and associated signalling pathways; *4,* the mesenchymal (M) and *5,* mesenchymal stem-like (MSL) subtypes characterized by an enrichment of Rho-mediated cell motility pathways, and enhanced cell differentiation pathways and interactions with extracellular matrix receptors; and *6,* a luminal androgen receptor (LAR)-expressing subtype that, while being ER-negative, has gene ontologies highly enriched in hormonal regulation and androgen receptor signal pathways [8]. While this more detailed TNBC taxonomy may lend itself to increased avenues in the search for targeted therapies, the incredible level of potential genetic heterogeneity within a single TNBC breast tumour (intra-tumour heterogeneity) can significantly limit the efficacy of current cytotoxic chemotherapies. In some cases, this could be because certain drugs are ineffective against a particular tumour cell subtype, enabling cell survival and the potential for metastasis. Once metastasis does occur, surgery and radiotherapy are no longer feasible options. Thus, a chemotherapeutic strategy that is less cytotoxic than routinely used chemotherapy agents but is still effective enough to target tumour cells irrespective of their gene profiles would be a major step forward in the battle against triple-negative breast cancer.

## 3. $H^+$ ions as a carcinogenic signal

In their seminal review, Hanahan and Weinberg [29] identified the unifying hallmarks of all cancers as their ability for: sustained proliferation, clonogenic replication, upregulation of tumour suppressor genes, resisting apoptosis, inducing angiogenesis, and initiating invasion and metastasis. Since then, the development of novel chemotherapies has aligned with targeting the molecular mechanisms underpinning many of the above traits. A decade later, the same authors updated the list adding that some, if not all, cancers were also defined by their ability to evade immune destruction and their capacity for reprogramming cellular metabolism to promote neoplastic proliferation [30]. This reprogramming involves a switch in glucose metabolism from the oxidative phosphorylation that occurs in normal cells to aerobic glycolysis in cancer cells, as was first observed by Otto Warburg [31,32]. Initially, it was thought that the hypoxic conditions within growing tumours limited the more energy efficient oxidative phosphorylation in favour of glycolysis, but this energy inefficient metabolic pathway still persists in cancer cells even when oxygen is present [33]. Indeed, the switch to aerobic glycolysis in leukemic and lung tumour cells occurs despite exposure to oxygen in the bloodstream and lungs respectively [34–37]. Commonly, though, hypoxic conditions can activate hypoxia-inducible factors 1 and 2 alpha (HIF-1α/2α) transcription factors that can independently upregulate glycolysis

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