Accepted Manuscript

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PII: S1043-6618(16)30014-7
DOI: http://dx.doi.org/doi:10.1016/j.phrs.2016.01.007
Reference: YPHRS 3033
To appear in: Pharmacological Research

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To appear in: Pharmacological Research

Received date: 7-1-2016
Accepted date: 10-1-2016
Are NHE1 and inducible nitric oxide synthase involved in human ovarian cancer?


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Gynaecological cancers associate with alterations in the synthesis of key molecules, including nitric oxide (NO), a gas involved in cell signalling modulating cell proliferation and metabolism [1]. The NO has a dual effect, acting as a tumour promoter or by preventing tumour growth in different types of cancer [1]. Intracellular alkalization and extracellular acidification result in increased NO synthesis by the NO synthase (NOS) isoform mainly expressed in endothelial cells (eNOS) leading to abnormal vascular reactivity [2]. The ability to alter the pH is a common characteristic of malignant cancer cells [3]. Tumour cells have an efficient capacity to acidify the extracellular pH (pHo) environment and to maintain an alkaline intracellular pH (pHi). Extracellular acidification facilitates tumour invasion and reduces immunesurveillance, a phenomenon that contributes to cancer malignity [4]. Thus, a role in the modulation of NO synthesis by mechanisms that control pHi or pHo could be crucial in gynaecological cancers, including human ovarian cancer.

Mammalian cells express different families of membrane transporters that control the content of acid-base equivalents generated as a consequence of their own metabolism. These transport systems modulate pHi by exporting intracellular acid equivalents to the extracellular space or endosomes, or by incorporating base equivalents [5, 6]. Protons (H+) are exported from the cells mainly by the sodium/H+ exchangers (NHEs) family of proteins in normal and cancer cells [6, 7]. Excessive generation of H+ and changes in pHo/pHi ratio could be detrimental to cell proliferation, survival, and metabolism, leading to a condition where cancer cells must adapt to proliferate and migrate [4]. In cells from normal tissues, the pH is maintained at physiological values reaching pHi ~7.0 (6.9 – 7.0) and pHo ~7.4 (7.3 – 7.4). However, these values are different in cancer cells, with pHi ~7.4 (7.1 – 7.7) and pHo ~6.6 (6.2–6.9), a phenomenon that results in higher cancer aggressiveness, invasion, and cellular metabolism [4, 7, 8]. Cervical cancer cells show overexpression of the NHE isoform 1 (NHE1), which associates with higher cell migration, and lower disease-free and overall survival of patients [9]. Thus, NHE1 could be considered as a prognostic factor in patients with cervical cancer. NHE1 was recently identified in primary cultures of human ovary tumour cells [10], but the regulatory mechanism(s) of its expression and activity has not yet been reported.
Alterations in the expression and activity of NOS are well described in patients with cancer. To date, expression of the inducible NOS (iNOS) isoform is upregulated and correlates with poor prognosis in breast cancer [11]. Other reports show that NO promotes human ovarian cancer cell growth and inhibits mitochondrial oxidative phosphorylation [12]. Thus, it is likely that increased NO availability will cause a glycolytic phenotype (i.e., Warburg effect), reducing mitochondrial respiration under an aerobic environment, leading to an increase in lactate generation and H+ release to the extracellular medium [12, 13]. Taking this in consideration the NO would also be a factor involved in the modulation of pH in cancer cells contributing to the generation of an acidic extracellular microenvironment. The latter leads to degradation of extracellular matrix promoting a pro-metastatic cellular behaviour. Therefore, NO will plays a role in metastasis and cancer cell migration by lowering the pHo in gynaecological cancers.

It is speculated that targeting NO synthesis could be a potential effective therapy against ovarian cancer [12]. This is based in the fact that iNOS expression is detected in ~50% of primary tumours and ~62% of metastatic lesions in human epithelial ovarian cancer [14]. The use of cisplatin, an alkylating agent used in chemotherapy [15], results in upregulation of iNOS, but downregulation of eNOS expression in human ovarian cancer, suggesting a key role of iNOS and/or iNOS/eNOS ratio in this type of gynaecological cancer.

Since in several human diseases the NOS activity and pH are altered and affect a variety of processes including tumour cells metabolism [1, 3, 4, 11, 12, 14], and expression and activity of H+ exchangers (mainly NHE1) are increased in tumour cells [4-8], we propose that human ovarian cancer cells will show a potential functional link between increase in NO synthesis and low pHo, but higher pHi (Figure 1). The role of NHE1 seems crucial in this phenomenon, which also generates a pro-inflammatory environment maintaining elevated iNOS expression and NO synthesis. Since higher NO level inhibits mitochondrial respiration and leads to higher H+ generation as a result of higher glycolytic metabolism, targeting NHEs and/or iNOS may be an approach to alter this potential tumour microenvironment vicious cycle in human ovarian cancer. Thus, a potential positive feedback loop maintained by the extracellular acidification and iNOS induction occurs in human ovarian cancer cells.
**Conflict of interest**

There is no conflict of interest.

**Author contribution**

Designed research study (CS, AL, DIC, FP, JG, LS), collected clinical data (AL, FP, EB), collected and analysed literature information (CS, JA, LN, EB, LT, MS, FT, JG, DIC, FP, AL, LS), designed the figure (CS, JA, LS), wrote the text (CS, LS).

**Acknowledgements**

This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT 3140516, 1150377, 1150344, 3160194, 11150083), Chile. EB holds a Faculty of Medicine, PUC-PhD fellowship, Chile. MS and LT hold Vicerectorate of Research, PUC-PhD fellowships, Chile.
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Figure 1. Potential functional link between NO and NHE1 in the extracellular medium acidification in human ovarian cancer. In human ovarian cancer cells the inducible nitric oxide synthase (iNOS) is overexpressed (†) leading to increased formation of nitric oxide (NO). The NO promotes (segmented black arrow) a glycolytic phenotype (Warburg effect) leading to H⁺ generation, which are removed via the sodium, proton (H⁺) exchanger isoform 1 (NHE1) reducing (\(\downarrow\)) the intracellular content of this acid equivalent. Extracellular accumulation of H⁺ contributes (green arrows) to the acidification of the extracellular space (Acidic), but alkalization of the intracellular space (Alkaline). Thus, a potential positive feedback loop or ‘vicious cycle’ maintained by the extracellular acidification and subsequent iNOS induction in proposed to occur in human ovarian cancer cells.