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Repositioning chloroquine and metformin to eliminate cancer stem cell traits in pre-malignant lesions

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ABSTRACT

Ideal oncology drugs would be curative after a short treatment course if they could eliminate epithelium-originated carcinomas at their non-invasive, pre-malignant stages. Such ideal molecules, which are expected to molecularly abrogate all the instrumental mechanisms acquired by migrating cancer stem cells (CSCs) to by-pass tumour suppressor barriers, might already exist. We here illustrate how system biology strategies for repositioning existing FDA-approved drugs may accelerate our therapeutic capacity to eliminate CSC traits in pre-invasive intraepithelial neoplasias. First, we describe a signalling network signature that overrides bioenergetics stress- and oncogene-induced senescence (OIS) phenomena in CSCs residing at pre-invasive lesions. Second, we functionally map the anti-malarial chloroquine and the anti-diabetic metformin ("old drugs") to their recently recognized CSC targets ("new uses") within the network. By discussing the preclinical efficacy of chloroquine and metformin to inhibiting the genesis and self-renewal of CSCs we finally underscore the expected translational impact of the "old drugs–new uses" repurposing strategy to open a new CSC-targeted chemoprevention era.

1. Introduction

An effective reduction of breast cancer mortality largely depends on our therapeutic ability to successfully intervene in the critical transition from non-invasive ductal carcinoma *in situ* (DCIS) to life-threatening invasive breast cancer (IBC). Our ever increasing knowledge of molecular and pathway biology in DCIS lesions can facilitate hypothesis-driven therapeutic strategies aimed to arrest invasion at the pre-malignant state of BC disease (Espina and Liotta, 2011). Because DCIS cells should adapt to survive in the highly stressful microenvironment of the intraductal niche (Gatenby and Gillies, 2008; Menendez and Lupu, 2007), they must circumvent hypoxia-induced apoptotic death while avoiding nutrient stress-induced senescence. Beyond evading biophysical constraints, DCIS cells must make use of alternative sources of energy such as the autophagic pathway, a major catabolic process that may permit DCIS starving cells to recycling intracellular components during periods of metabolic stress to maintain homeostasis and viability (Lum et al., 2005; Mathew et al., 2007). Remarkably, this metabolic adaptation appears to occur in DCIS tumour-founding progenitor cells because pre-malignant, cytogenetically abnormal DCIS spheroid-forming cells directly isolated from human DCIS lesions have increased expression of autophagy-associated proteins that persist in culture and in tumours generated by these cells in immunosupressed NOD/SCID mice (Espina et al., 2010). Given that: (a) the anti-autophagy small-molecule chloroquine has been found to kill DCIS progenitor spheroids and prevent their tumorigenicity in mice *via* reduced expression of autophagy-associated proteins (Espina et al., 2010) and (b) the BC invasive phenotype is already genetically programmed at pre-invasive stages of disease progression (*i.e.* DCIS lesions), pharmacological abrogation of autophagy may be viewed as a novel therapeutic strategy for BC chemoprevention. This scenario strongly supports the Preventing Invasive Neoplasia with Chloroquine (PINC) trial (NCT01023477), which will measure the effectiveness of chloroquine administration to patients with low-grade, intermediate-grade or high-grade DCIS to directly test the hypothesis that pharmacological blockade of autophagy is an effective treatment for DCIS (Espina and Liotta, 2011).

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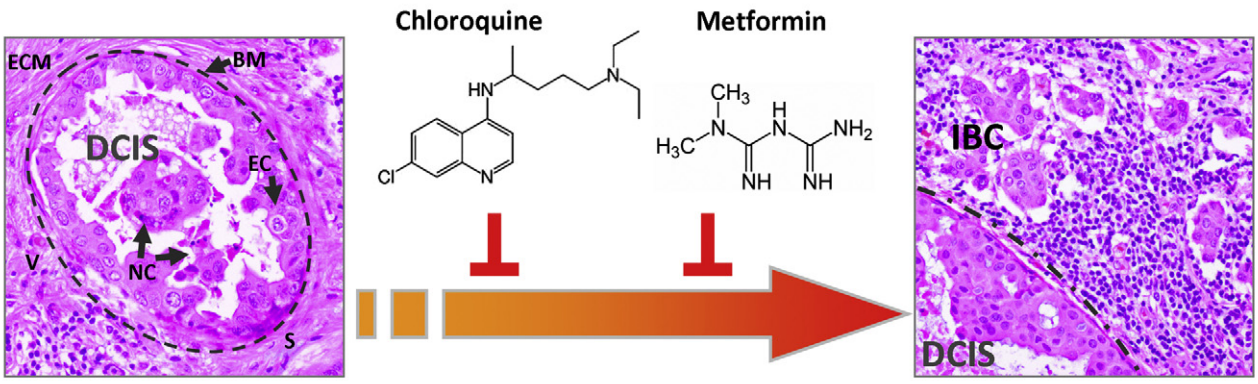

Fig. 1. Prevention and treatment of pre-malignant lesions for accelerated development of existing anti-CSC drugs. Cell adaptation to chronic stressful conditions that occur in oxygen- and nutrient-starved areas of pre-malignant DCIS lesions (*left*) could lead to the generation of CSC within the mass of cells accumulating in the duct before the onset of BC invasion (*right*). Repositioning pre-existing drugs such as chloroquine and metformin to molecularly impede all the instrumental mechanisms acquired by migrating CSCs to by-pass tumour suppressive barriers may open a new chemoprevention era aimed to curatively inhibit the genesis and self-renewal of CSCs (BM: basement membrane – dashed line; EC: epithelial cells; ECM: extracellular matrix; IBC: invasive breast cancer; S: stroma; V: blood vessels).

Developing a known drug (*e.g.* chloroquine, which is the drug of choice used for the prophylaxis treatment of malaria because it is effective, low toxic to humans, and inexpensive) for another clinical purpose (*e.g.* prevention of the invasive progression of pre-malignant lesions such as DCIS in BC) is termed *repositioning* or *repurposing*. This approach can be very effective to develop new oncology therapeutics since many existing drugs have been studied for their pharmacokinetics and safety profiles and often have already been approved by the regulatory agencies FDA (US), EMEA (Europe) and MHLW (Japan). Here, we exemplify how system biology strategies for repositioning regulatory agencies-approved drugs chloroquine and metformin may accelerate our therapeutic capacity to prevent invasive progression of pre-invasive intraepithelial neoplasias by eliminating cancer stem cell (CSC) traits (Fig. 1). First, we illustrate a signalling network signature that CSCs should necessarily acquire to successfully override intrinsic tumour suppressor barriers (*e.g.* metabolic- and oncogene-induced senescence) activated in pre-malignant lesions. Second, we functionally map the anti-malarial chloroquine and the anti-diabetic metformin (the "old drugs") to their presumed CSC molecular targets (the "new uses") within the network: chloroquine, by inhibiting autophagy, is expected to impede a crucial manner of energy production that allows CSCs to survive hypoxic and nutrient-deprived microenvironments. Metformin, by preventing the molecular transition of epithelial tumour cells to embryonic mesenchymal phenotypes (EMT), is expected to block an essential senescence escape mechanism while nullifying EMT-driven CSC features. We finally discuss the preclinical efficacy of the repositioned drugs to inhibiting the genesis and self-renewal of CSCs, thus underscoring the translational impact of the "old drugs–new uses" repurposing strategy, which may rapidly provide us with ideal, curative oncology drugs able to arrest epithelium-originated carcinomas at their non-invasive, pre-malignant stages.

2. Autophagy and oncogene-induced senescence (OIS): more than friends

Besides biophysical stress-induced senescence, DCIS lesions should circumvent also oncogene-induced senescence (OIS) (Braig and Schmitt, 2006; Collado and Serrano, 2010; Serrano, 2010) before they develop into IBC. Permanent activation of certain oncogenic pathways causes cell senescence by default and many human transformed cells, before reaching full malignancy, stop proliferating and undergo senescence at the pre-malignant (non-invasive) stage, at which senescence-inducing signals (*e.g.* oncogenic proteins, oxidative stress, persistent DNA damage) reach sufficient intensity to be effective (Braig and Schmitt, 2006; Collado and Serrano, 2010; Serrano, 2010). Proliferating IBC cells with activated oncogenes, therefore, truly represent progeny of tumour cells that have acquired mechanisms to suppress OIS in earlier stages of BC pathogenesis (*e.g.* DCIS). In this regard, landmark studies have revealed that autophagy is a causal pre-requisite for senescence (Young and Narita, 2010) and, accordingly, interference with autophagy impedes stress-induced senescence and significantly attenuates the extent of OIS. This scenario might appear to contradict a recent suggestion that autophagy is a main determinant of DCIS cell fate because it allows DCIS cells to survive and proliferate by evading cell cycle arrest/senescence responses to the high-stress microenvironment of the intraductal space (Espina et al., 2010). In fact, although autophagy does contribute to tumour suppression by actively controlling the senescence phenotype, if tumour cells somehow bypass OIS, autophagy would then help such cells to survive DCIS-associated biophysical stresses, unwittingly facilitating their full transformation. A causal relationship between autophagy and cell survival in DCIS lesions has been illustrated by the fact that ATG6/Beclin-1 (a haploinsufficient tumour suppressor protein that is essential for autophagy (Karantza-Wadsworth et al., 2007; Liang et al., 1999)) is upregulated in human comedo-DCIS at the viable rim of intraductal cells within the hypoxic ductal niche. On the contrary, deletion or monosomy of the *ATG6/Beclin-1* gene significantly associates with *ERBB2* oncogene amplification (both on 17q21) in a subset of BC (Negri et al., 2010). Because *ERBB2* overexpression can be found in ~25% of invasive/metastastic BC, but it takes place in 50–60% of DCIS in general and 60–70% of high-grade DCIS, these findings altogether raise the interesting question about whether an enhanced autophagic flux, while indispensable in terms of DCIS cell survival (Espina and Liotta, 2011; Espina et al., 2010; Gatenby and Gillies, 2008; Lum et al., 2005; Mathew et al., 2007; Menendez and Lupu, 2007), is also necessary and/or sufficient to promote progression from non-invasive to IBC in different cell origin subtypes of DCIS (Hannemann et al., 2006; Muggerud et al., 2010).

2.1. Loss of autophagic genes: a chance to escape from the senescence prison

The apparent paradox that a metastasis-promoting oncoprotein (*e.g. ERBB2*) is more frequently overexpressed in non-invasive DCIS than in IBC is consistent with the prevailing view that a single oncogene is insufficient to drive IBC progression. Because *ERBB2* can be expected to trigger stress-induced premature senescence and