Targeting Breast Stem Cells with the Cancer Preventive Compounds Curcumin and Piperine

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Keywords: Breast stem cells, cancer prevention, curcumin, piperine

Abstract

The cancer stem cell hypothesis asserts that malignancies arise in tissue stem and/or progenitor cells through the dysregulation or acquisition of self-renewal. In order to determine whether the dietary polyphenols, curcumin, and piperine are able to modulate the self-renewal of normal and malignant breast stem cells, we examined the effects of these compounds on mammosphere formation, expression of the breast stem cell marker aldehyde dehydrogenase (ALDH), and Wnt signalling. Mammosphere formation assays were performed after curcumin, piperine, and control treatment in unsorted normal breast epithelial cells and normal stem and early progenitor cells, selected by ALDH positivity. Wnt signalling was examined using a Topflash assay. Both curcumin and piperine inhibited mammosphere formation, serial passaging, and percent of ALDH+ cells by 50% at 5 μM and completely at 10 μM concentration in normal and malignant breast cells. There was no effect on cellular differentiation. Wnt signalling was inhibited by both curcumin and piperine by 50% at 5 μM and completely at 10 μM. Curcumin and piperine separately, and in combination, inhibit breast stem cell self-renewal but do not cause toxicity to differentiated cells. These compounds could be potential cancer preventive agents. Mammosphere formation assays may be a quantifiable biomarker to assess cancer preventive agent efficacy and Wnt signalling assessment can be a mechanistic biomarker for use in human clinical trials.

Introduction

The cancer stem cell hypothesis asserts that malignancies arise in tissue stem and/or progenitor cells through the dysregulation or acquisition of self-renewal [1]. Stem cells are long lived and capable of acquiring multiple mutations over time to transform to malignancy, while differentiated cells turn over rapidly [2]. Clonal expansion of stem cell populations through dysregulated self-renewal is hypothesized to be an early step in carcinogenesis [3]. This hypothesis is supported by recent studies demonstrating that breast tissue from women who carry germline BRCA1 mutations contains islands of cells that uniformly express the stem cell marker ALDH1. These expanded stem cell colonies display loss of heterozygosity for the normal BRCA1 allele [4, 5]. Experimental knockdown of BRCA1 in normal mammary cells leads to an increase in the ALDH-positive stem cell population *in vitro* and in mouse models [5].

If primitive breast cells are the targets for transforming events, then interventions aimed at reducing this cell population may provide novel risk reduction strategies. Current strategies, which include prophylactic mastectomy for BRCA1 or BRCA2 carriers, as well as the anti-oestrogens tamoxifen and raloxifene, are all associated with potential toxicities. These toxicities have limited the widespread utilization of tamoxifen and raloxifene in cancer prevention [6, 7]. Furthermore, hormonal interventions selectively prevent estrogen receptor (ER) positive breast cancers, but are less effective, and may actually increase the development of ER negative breast cancers [8–10]. This highlights the important need to develop non-toxic strategies to effectively prevent both ER negative and ER positive breast cancer. If the cancer stem cell hypothesis is valid, then strategies aimed at targeting stem cell self-renewal pathways represent rational approaches for cancer prevention. One such pathway is the Wnt signalling pathway, which is dysregulated in breast cancer, as well as many other malignancies [11–13]. Although the development of specific pharmacologic Wnt inhibitors has proven a challenge, there is evidence that curcumin, a dietary polyphenol found in spices, is able to downregulate the Wnt signalling pathway [14]. Interestingly, there is substantial evidence in preclinical models that curcumin is a potent chemopreventive dietary agent [15–19]. This suggests that the protective effects of curcumin might be due to Wnt inhibition of self-renewal in breast stem/progenitor cells.