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Curcumin inhibits development and cell adhesion in *Dictyostelium discoideum*: Implications for YakA signaling and GST enzyme function

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ABSTRACT

The molecular basis for nutraceutical properties of the polyphenol curcumin (*Curcuma longa*, Turmeric) is complex, affecting multiple factors that regulate cell signaling and homeostasis. Here, we report the effect of curcumin on cellular and developmental mechanisms in the eukaryotic model, *Dictyostelium discoideum*. *Dictyostelium* proliferation was inhibited in the presence of curcumin, which also suppressed the prestarvation marker, discoidin I, members of the *yakA*-mediated developmental signaling pathway, and expression of the extracellular matrix/cell adhesion proteins (DdCAD and csA). This resulted in delayed chemotaxis, adhesion, and development of the organism. In contrast to the inhibitory effects on developmental genes, curcumin induced *gstA* gene expression, overall GST activity, and generated production of reactive oxygen species. These studies expand our knowledge of developmental and biochemical signaling influenced by curcumin, and lends greater consideration of GST enzyme function in eukaryotic cell signaling, development, and differentiation.

1. Introduction

Curcumin (*Curcuma longa*, Turmeric), a plant polyphenol derived from turmeric root, is recognized for its anti-carcinogenic, anti-inflammatory, and therapeutic effects. Because of its multivalent complexity, curcumin wields pleiotropic effects that directly or indirectly modulate biological processes [1]. Curcumin non-selectively inhibits proliferation of normal (non-malignant) cells [2,3] and can alter cell cycle control mechanisms [4]. It also modifies expression and activity of downstream effectors within the signal transduction cascade [5], induces non-inflammatory phagocytosis in macrophages [6], blocks neutrophils [7], and inhibits migration of retinal endothelial cells [8]. *In vitro* and *in vivo* treatment with curcumin and other flavonoids influence pro- and anti-oxidant conditions within cells, which is linked to activation and/or inhibition of phase I (cytochrome P450) and phase II (glutathione *S*-transferase, GST) metabolizing enzymes [1,9,10,11]. GST enzymes function to regulate the solubility and/or conjugation of various substrates within cells and tissues, and altered GST activity has been linked to the therapeutic effects of curcumin and other plant polyphenols [12–15]. GST enzymes catalyze the conjugation of GSH (glutathione) with a variety of electrophilic compounds to effect detoxification, cell signaling, metabolism, and homeostasis. *In silico* modeling postulates that curcumin may bind directly to GST enzymes [16], suggesting that curcumin influences cell functions through GST-mediated metabolism.

The eukaryotic model organism *Dictyostelium discoideum* when starved, secretes cAMP and undergoes chemotaxis to form multicellular aggregates. *Dictyostelium* YakA, a tyrosine-related kinase expressed during starvation, regulates developmental signaling, induces the expression of chemotactic and developmental mediators such as PKA-C, ACA, and cAR1 [17], and secretion of differentiation [18] and cell-adhesion factors [19,20] that underlie aggregate formation, which then proceeds through stage-specific morphogenesis that produces a mature fruiting body.

Recent studies demonstrate that *Dictyostelium* phase I cytochrome P450 oxidoreductase (*redA*) *redA* mutants are arrested at the mound stage of development [21]. *Dictyostelium* genome sequencing reveals five GST transcripts that with 35–40% (amino acid) similarity with human GST alpha. Thus, expression of CYPs and GSTs in *Dictyostelium* present new opportunities to elucidate their role(s) in eukaryotic cell signaling, homeostasis, and development [22,23]. While some studies have characterized the effect of polyphenols on *Dictyostelium* growth, little is known about the impact of curcumin, or plant polyphenols on phase II GST expression and activity in the organism.

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This study characterized the effect of curcumin on the growth and development of *Dictyostelium*. We report that curcumin inhibition of proliferation and development is dose-dependent, down-regulating key members of the *yakA* signaling pathway. Curcumin exposure compromised expression of extracellular matrix, and cytoskeletal network proteins that underlie properties of cellcell adhesion. In addition, curcumin exposure selectively up-regulates two of the five *gstA* transcripts that are expressed during starvation, and induces GST activity. We provide evidence that curcumin generates the production of reactive oxygen species that may also impact YakA signaling and GST regulation. These studies expand our knowledge of biochemical pathways affected by curcumin, and expand consideration for significance of GST enzymes in eukaryotic cell growth, signaling, and development.

2. Materials and methods

2.1. Proliferation

Dictyostelium AX4 (axenic) amoebae were cultured at 22 °C on rotary shaker (180 rpm) in HL5 axenic media containing 100 μg/mL of streptavidin and 100 units/mL penicillin. Curcumin (E,E)-1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, (Sigma Chemical Co.) was prepared in ethanol and added to 5×10^4 cells/mL in HL5 with 2.5, 5, 10, and 20 μM curcumin. Aliquots were removed and cell counts obtained by hemocytometer. Trypan blue exclusion was used for cell viability and integrity. Axenic control and curcumin treated cells were used for development, chemotaxis, and biochemical assays described below.

2.2. Chemotaxis

Log phase axenic cells were grown at 2×10^6 cells/mL in HL5 media with and without curcumin. After 24 h, the cells were centrifuged, washed twice in KK2 buffer, and resuspended in KK2 buffer (20 mM potassium phosphate, pH 6.2, 2 mM $MgCl_2$) at a concentration of 2×10^8 cells/mL. Samples were pulsed every six minutes with 50 nM cAMP for five hours. Chemotaxis assays were performed as previously [24], with 50 μM cAMP as the target destination.

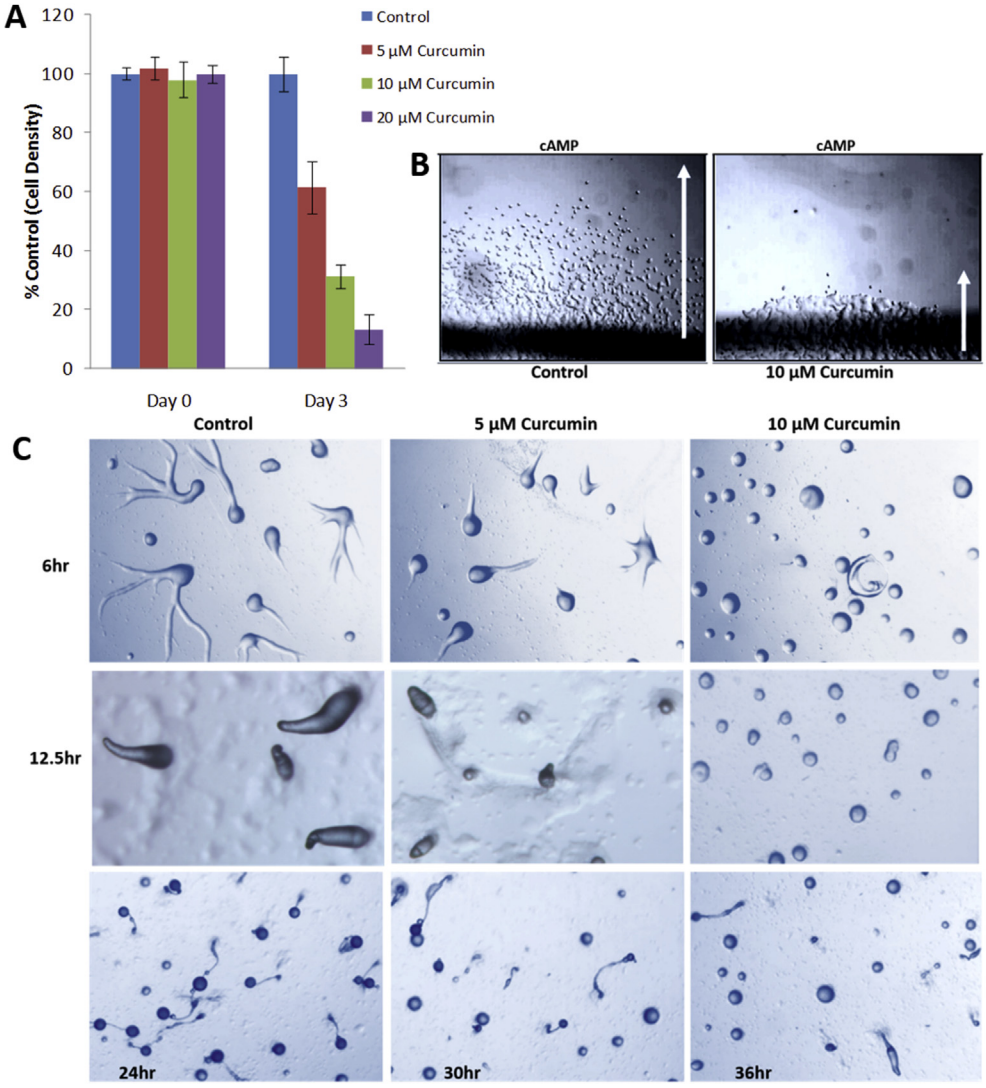

Fig. 1. Effect of curcumin on AX4 proliferation. (A) Anti-proliferative were seen with 5, 10, and 20 μM concentrations and was maximized by 72 h. (B) Cells grown in the presence of 10 μM curcumin exhibit delayed chemotaxis/migration toward a 50 μM cAMP source as monitored over a 3hr period. Histograms and images of at least three experiments (n = 3) performed in triplicate. (C) In comparison to controls, differences in curcumin treated cells is evident by12.5 h; controls display tipped aggregate/pre-slug phenotypes, whereas curcumin produced small tipped aggregates or only aggregates. Controls produced fruiting bodies by 24 h; curcumin treated amoebae manifested limited fruiting bodies at 30 h and 36 h of development.