EXPERIMENTAL CELL RESEARCH 317 (2011) 82–93

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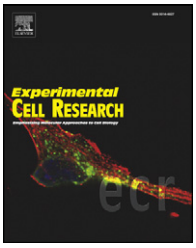

Research Article

Induction of murine embryonic stem cell differentiation by medicinal plant extracts

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ARTICLE INFORMATION

Article Chronology:
Received 2 July 2010
Revised version received
27 August 2010
Accepted 9 October 2010
Available online 15 October 2010

Keywords:
Rex-1
Nanog
Oct-4
Suriana maritima
Simarouba glauca
Quassia africana

ABSTRACT

Epidemiological evidence indicates that diets high in fruits and vegetables provide a measure of cancer chemoprevention due to phytochemical constituents. Natural products are a rich source of cancer chemotherapy drugs, and primarily target rapidly cycling tumor cells. Increasing evidence indicates that many cancers contain small populations of resistant, stem-like cells that have the capacity to regenerate tumors following chemotherapy and radiation, and have been linked to the initiation of metastases. Our goal is to discover natural product-based clinical or dietary interventions that selectively target cancer stem cells, inducing differentiation. We adapted an alkaline phosphatase (AP) stain to assay plant extracts for the capacity to induce differentiation in embryonic stem (ES) cells. AP is a characteristic marker of undifferentiated ES cells, and this represents a novel approach to screening medicinal plant extracts. Following a survey of approximately 100 fractions obtained from 12 species of ethnomedically utilized plants, we found fractions from 3 species that induced differentiation, decreasing AP and transcript levels of pluripotency markers (Nanog, Oct-4, Rex-1). These fractions affected proliferation of murine ES, and human embryonal, prostate, and breast carcinoma cells in a dose-dependent manner. Several phytochemical constituents were isolated; the antioxidant phytochemicals ellagic acid and gallic acid were shown to affect viability of cultured breast carcinoma cells.

Introduction

There is increasing evidence that many cancers contain small populations of pluripotent cells capable of propagating tumors following treatment with radiation or cytotoxic chemotherapy drugs [1]. First identified in leukemia, cancer stem cells (CSCs) have since been discovered in many solid tumors, including breast, ovary, brain, melanoma, multiple myeloma, pancreas, head and neck, colon, and lung [2,3]. It is believed that these cells have the ability to both self-renew and differentiate into tumor cells, leading to a hierarchical model that may explain the cellular heterogeneity found in many tumors. Resistance to traditional

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Abbreviations: AP, alkaline phosphatase; AP-1, activator protein 1; AR, androgen receptor; CSC, cancer stem cell; DMSO, dimethyl sulfoxide; ER, estrogen receptor; ES, embryonic stem; EtOAc, ethyl acetate; etOH, ethanol; HIF-1α, Hypoxia-inducible factor-1α; LIF, leukemia inhibitory factor; LXR, liver X receptor; MeOH, methanol; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; PI, propidium iodide; QA, aqueous fraction of leaf extracts from *Q. africana*; RA, *all-trans* retinoic acid; RXR, retinoid X receptor; SG, BuOH fraction of wood extract from *S. glauca* saplings; SM, etOAc fraction of leaf and stem extracts from *S. maritima*; VC, vehicle control; WCMC, Weill Cornell Medical College

0014-4827/$ – see front matter
doi:10.1016/j.yexcr.2010.10.010

Table 1 – Plant species screened in cell viability and alkaline phosphatase assay. Species were separated into plant parts and extracted in methanol. Following the removal of the methanol, extracts were suspended in water and partitioned with hexane, ethyl acetate, and butanol to make 4 distinct fractions (including aqueous) for each plant part. Approximately 100 fractions were initially obtained and assayed for alkaline phosphatase activity and cytotoxicity.

Family	Species	Parts used
Fabaceae	*Lotononis bainesii*	Aerial parts, roots
Fabaceae	*Sutherlandia frutescens*	Seeds, leaves
Simaroubaceae	*Simarouba glauca*	Fruit pulp, seeds, roots, leaves, stem, bark
Vitaceae	*Cissus quadrangularis*	Stems
Vitaceae	*Cissus sp.*	Leaves and stems
Simaroubaceae	*Simarouba amara*	Leaves, twigs
Irvingiaceae	*Klainedoxa gabonensis*	Leaves
Simaroubaceae	*Quassia africana*	Leaves
Surianaceae	*Suriana maritima*	Leaves and stems
Irvingiaceae	*Irvingia gabonensis*	Leaves
Asteraceae	*Artemisia annua*	Aerial parts
Rutaceae	*Murraya koenigii*	Inflorescences, leaves and stems
Orchidaceae	*Dendrobium chrysotoxum*	Stem, flowers

cancer chemotherapeutic drugs via the proliferation of these CSCs may also explain cancer recurrence after treatment and the subsequent initiation of metastases.

Normal stem cells are exposed to a high number of mutagenic events over time, which may in turn lead to the initiation and progression of carcinogenesis from somatic stem cells or progenitor cells [1]. The dysregulation of signaling pathways such as Notch, Shh, and Wnt contributes to self-renewal and can lead to oncogenesis [4]. Many CSCs exhibit characteristics of normal stem cells, including self-renewal, anchorage-independent growth, high cloning efficiency, and the expression of anti-apoptotic and transporter proteins [5]. Strategies that target CSCs represent a new model for cancer chemoprevention and treatment, one that aims to prevent CSCs from continuing to drive tumor growth.

Most of the currently available pharmaceutical drugs for cancer are derived from natural products [6]. Phytochemicals target a variety of cancer transcription factors such as Sp1, Sp3, c-Myc, hypoxia-inducible factor 1α (HIF-1α), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and activator protein 1 (AP-1) [7–9]. Quassinoids, polyphenolics, triterpenoids, lignans, and diterpene esters have demonstrated an ability to induce differentiation, downregulate c-Myc, and affect apoptotic pathways in stem-like cancer cells through a wide array of mechanisms [10–15].

The signaling molecule all-*trans* retinoic acid (RA) is currently used as a differentiation-inducing drug in combination with arsenic trioxide to successfully treat acute promyelocytic leukemia [16]. However, there are no pharmaceutical drugs currently in use for differentiation therapy against solid tumors. As the body of literature that describes and identifies CSCs grows, there is a corresponding interest in the discovery of agents that affect pluripotency and drive differentiation. Our goal is to discover natural product-based clinical or dietary interventions that

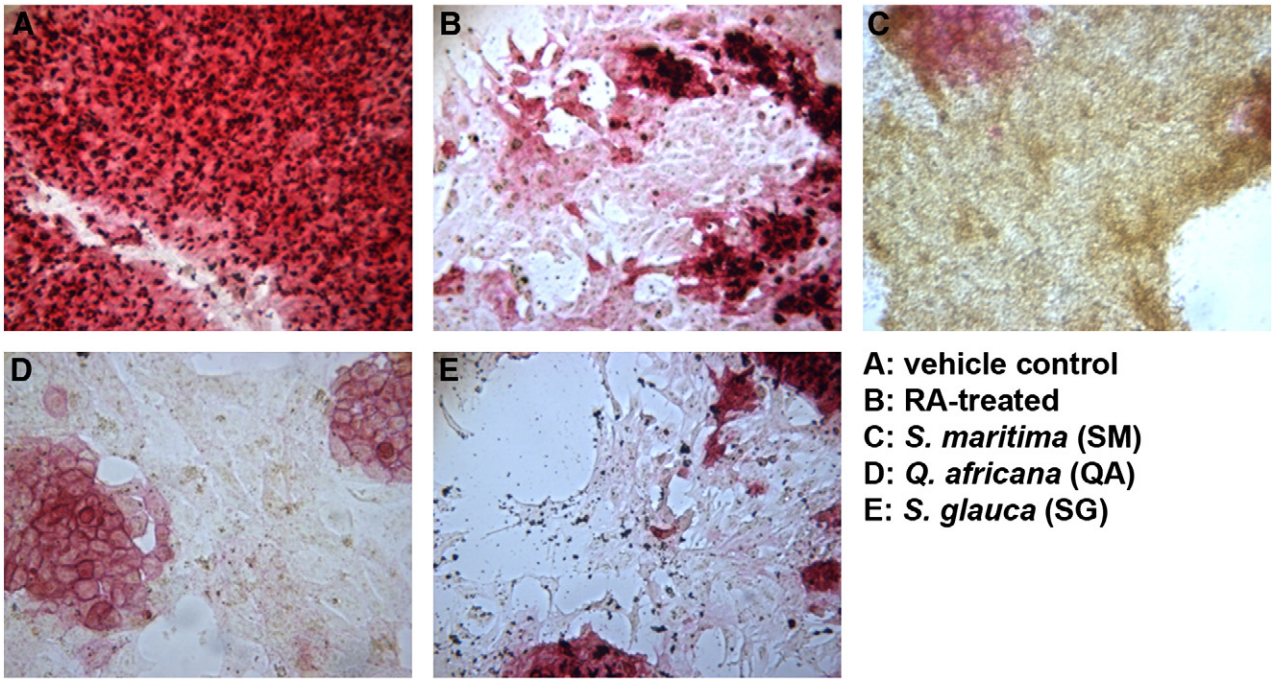

Fig. 1 – Alkaline phosphatase staining of murine embryonic stem cells. Cells were treated with semi-purified fractions of plant extracts. Fractions were dried completely, resuspended in DMSO, and diluted in culture medium to final concentrations (1, 5, 10 and 50 μg/ml) and a DMSO concentration ≤0.1%. After 96 h treatment, cells were stained and compared to (A) vehicle control (0.01% DMSO) and (B) 1 μM RA treatment. Alkaline phosphatase activity decreases rapidly with the onset of differentiation, which can be seen in B–E. Pictured are cells treated with (C) the ethyl acetate fraction of leaf and stem extracts of *S. maritima* (50 μg/ml); (D) the aqueous fraction of leaf extracts from *Q. africana* (50 μg/ml); and (E) the butanol fraction of wood extract from *S. glauca* saplings (5 μg/ml). Experiments were performed in duplicate wells with at least 3 independent biological replicates. Color images can be found online.