Evaluation of aqueous extracts of *Taraxacum officinale* on growth and invasion of breast and prostate cancer cells

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Abstract. Ethnotraditional use of plant-derived natural products plays a significant role in the discovery and development of potential medicinal agents. Plants of the genus *Taraxacum*, commonly known as dandelions, have a history of use in Chinese, Arabian and Native American traditional medicine, to treat a variety of diseases including cancer. To date, however, very few studies have been reported on the anti-carcinogenic activity of *Taraxacum officinale* (TO). In the present study, three aqueous extracts were prepared from the mature leaves, flowers and roots, and investigated on tumor progression related processes such as proliferation and invasion. Our results show that the crude extract of dandelion leaf (DLE) decreased the growth of MCF-7/AZ breast cancer cells in an ERK-dependent manner, whereas the aqueous extracts of dandelion flower (DFE) and root (DRE) had no effect on the growth of either cell line. Furthermore, DRE was found to block invasion of MCF-7/AZ breast cancer cells while DLE blocked the invasion of LNCaP prostate cancer cells, into collagen type I. Inhibition of invasion was further evidenced by decreased phosphorylation levels of FAK and src as well as reduced activities of matrix metalloproteinases, MMP-2 and MMP-9. This study provides new scientific data on TO and suggests that TO extracts or individual components present in the extracts may be of value as novel anti-cancer agents.

Introduction

Plants of the genus *Taraxacum*, also known as dandelions, are members of the Asteraceae family. These perennial plants are widespread throughout the warmer temperate zones of the Northern Hemisphere and have been used for centuries as a remedy for various ailments by several societies. Dandelions play a pivotal role in traditional Chinese medicine (TCM) and are frequently used for treatment of breast, uterine and lung tumors as well as hepatitis and digestive diseases (1,2), while Native Americans use dandelion roots and herbs to treat kidney disease, dyspepsia and heartburn. In traditional Arabian medicine, dandelions have been applied to remedy liver and spleen disorders (3), whereas European herbalists authorize the use of dandelions for fever, boils, eye problems, diabetes and diarrhea (4).

The variety of health benefits associated with the use of dandelions has been attributed to specific *Taraxacum* species as extracts of the whole plants or specific plant parts. Anti-carcinogenic activities have been reported for the aqueous root extract of *Taraxacum japonicum* on mouse skin tumors (5). Further study revealed that taraxasterol and taraxerol, triterpenoids isolated from *T. japonicum*, were responsible for the observed effect on mouse skin tumors and that taraxasterol inhibited spontaneous mammary carcinogenesis, after oral administration (6). Taraxinic acid, isolated from *Taraxacum coreanum* showed potent antiproliferative activity against HL-60 cells (7) and the ethanolic extracts of the Chinese dandelion root (*Taraxacum mongolicum*) inhibited the growth of B16 2F2 mouse melanoma cells (8). Antitumor action has also been demonstrated for aqueous *Taraxacum officinale* (TO) extracts, through TNF-α and IL-1α secretion in HeP G2 cells (9).

Based on the limited availability of scientific data on the effect of *Taraxacum* species on human breast cancer and the claims of the use of the common dandelion, *Taraxacum*
Table I. % Yield obtained after cold aqueous extraction of 75 g of dried plant material.

<table>
<thead>
<tr>
<th>Taraxacum officinale</th>
<th>Before (g)</th>
<th>After (g)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowers</td>
<td>75</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Leaves</td>
<td>75</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Roots</td>
<td>75</td>
<td>12</td>
<td>16</td>
</tr>
</tbody>
</table>

officinale, to treat prostate cancer, we examined whether dried aqueous extracts of various parts of Taraxacum officinale have an effect on MCF-7/AZ breast and LNCaP C4-2B prostate cancer cells. In the current, study we have analyzed the impact of the different TO extracts on proliferation and metastasis related properties.

This study demonstrates a number of previously unknown effects of Taraxacum officinale on human cancer cells and suggests that TO extracts or individual components present in the extracts may be of value as novel anti-cancer agents.

Materials and methods

Plant materials and preparation of extracts. Plant materials of Taraxacum officinale were obtained from local herb stores. Voucher samples are archived in the laboratory and are available for analysis by contacting the corresponding author. The cold aqueous extracts were prepared as follows: 75 g of the dried plant parts were soaked in water for 24 h at room temperature. The mixtures were filtered to remove particulate matter, lyophilized and the resulting powders were stored in a desiccator at 4°C. Table I shows the yields obtained from the different parts.

Antibodies and other reagents. Antibodies directed to p-FAK (Tyr397) and p-src (Tyr418) were from Invitrogen (Carlsbad, CA). Mouse anti-FAK and anti-src monoclonal antibodies (mAb) were from Transduction Laboratories (San Jose, CA). Rabbit anti-ERK and anti-p-ERK (Thr202/Tyr204) were obtained from Cell Signaling Technologies (Beverly, MA). Secondary biotinylated anti-rabbit and anti-mouse Abs and Vectastain® ABC-AmP™ kit were from Vector Laboratories (Burlingame, CA). ET-18-OOMe (clinical grade) was kindly provided by Dr P. Hilgard (ASTA Medica, Frankfurt am Main, Germany). Drug toxicity was evaluated through measurement of mitochondrial dehydrogenase activities with MTT reagent (Sigma, St. Louis, MO) (10). BCA (bicinchoninic acid) protein assay reagent kit was from Pierce Biotechnology (Rockford, IL).

Cell culture. MCF-7/AZ is a variant of the human mammary carcinoma cell family MCF-7 (11). The cells were maintained at 37°C in a mixture of Dulbecco's modified Eagle's medium (DMEM) and HAMF12 (50/50) supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% fetal bovine serum (FBS) (Invitrogen), in a humidified atmosphere containing 10% CO2. The metastatic prostate cancer subline LNCaP C4-2B (12,13) was grown in RPMI-medium supplemented with 5% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin (Invitrogen) at 37°C in a humidified atmosphere containing 5% CO2.

Assay for cell viability. Cell viability was tested in accordance with Romijn et al. (10). Briefly, mitochondrial dehydrogenase activities were measured by an MTT-reagent (Sigma). Cells were seeded in microtiter plates at an initial density of 1.5x10⁴ cells in 200 μl culture medium and treated with increasing concentrations of each TO extract. In each experiment, eight wells were used to determine the mean O.D. referring to cell viability.

Assays for cell growth Sulforhodamine B assay. Cells were seeded in microtiter plates at an initial density of 1.5x10⁴ cells in 200 μl culture medium and treated with increasing concentrations of each TO extract. After an incubation period of 4 days, the amount of cell protein in each well was estimated with the sulforhodamine B assay (SRB) (Sigma) (14).

Cell counting. Cells were seeded in 25-cm² culture flasks at a density of 1.5x10⁵ cells in 5 ml. The cells were grown in the presence or absence of the crude aqueous TO extracts for 4 days, and counted with a hemacytometer (Hauser Scientific, Horsham, PA).

Collagen type I invasion assay. Six-well plates were filled with 1.25 ml neutralized type I collagen (0.09%, Millipore, Billerica, MA) and incubated for 1 h at 37°C to allow gelification. For invasion into collagen type I gels, non-invasive MCF-7/AZ cells, pretreated with ET-18-OOMe for 24 h to become invasive into collagen type I (15) and LNCaP C4-2B cells, were harvested using Moscona buffer and trypsin/EDTA and seeded on top of collagen type I gels. Cultures were incubated for 24 h at 37°C in the presence or absence of the different TO extracts. Numbers of cells penetrating into the gel or remaining at the surface were counted, using an inverted microscope and expressed as the invasion index, being the percentage of invading cells over the total number of cells (16).

Western blotting. Cell lysates were made from 70% confluent cultures. Cells were treated for the indicated times with the crude aqueous TO extracts, washed and lysed in 0.5 ml lysis buffer containing 1% Triton X-100, 1% NP-40 and the following inhibitors: aprotonin (10 μg/ml), leupeptin (10 μg/ml), PMSF (1.72 mM), NaF (100 mM), NaVO₄ (500 μM) and Na₃P₂O₇ (500 μg/ml). Aliquots of lysates containing the same quantity of proteins were boiled for 5 min in SDS-PAGE sample buffer supplemented with 5% ß-mercaptoethanol, electrophoresed on 7.5% SDS-PAGE and transferred to PVDF membranes (Immobilon-P, Bio-Rad Laboratories, Hercules, CA). After transfer, membranes were incubated with relevant antibodies vs p-FAK, p-src and p-ERK followed by incubation with a secondary biotinylated antibody and developed by ECL, using the Vectastain® ABC-AmP™ detection kit. The membranes were stripped at 50°C for 30 min in 100 mM ß-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl (pH 6.8),
Table II. O.D.₈₀ values for each crude aqueous TO extract as determined by MTT assay after 24 h exposure.

<table>
<thead>
<tr>
<th>Taraxacum officinale</th>
<th>MCF-7/AZ</th>
<th>LNCaP C4-2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowers</td>
<td>&gt;500</td>
<td>30</td>
</tr>
<tr>
<td>Leaves</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Roots</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

**Results**

**Effect of Taraxacum officinale extracts on cell viability.** MCF-7/AZ breast cancer cells and LNCaP C4-2B prostate cancer cells were exposed to increasing concentrations of DFE, DLE and DRE for 24 h. The MCF-7/AZ cell viability was unaffected (98.2±1.4%) by the extract of the flower at concentrations up to 500 μg/ml, while the percentage of living LNCaP C4-2B cells significantly dropped to 60.5±2.1% at 50 μg/ml. The extracts of the flower and the root equally affected both cell lines, with DRE being more toxic than DLE, leaving only 50% viable at a concentration of 50 μg/ml as compared to 180 μg/ml of the leaf extract (data not shown). For further experiments O.D.₈₀ values were used, leaving approximately 80% of the cells viable (Table II).

**Inhibition of ERK and cell growth in human breast cancer cells.** The aqueous DLE markedly inhibited the growth of MCF-7/AZ breast cancer cells, by 40%, after 96 h treatment and this in a concentration-dependent manner (Fig. 1A and B), while no significant effect could be observed on the growth of LNCaP C4-2B cells. DFE and DRE did not affect the growth of either cell line (Fig. 1A). As shown in Fig. 1C, exposure of MCF-7/AZ cells to DLE resulted in a time-dependent decrease of ERK activities as compared to untreated controls, whereas no changes in phosphorylation levels of ERK were observed when cells were exposed to DFE and DRE (data not shown). Total levels of ERK were unaltered upon exposure (data not shown).

**Anti-invasive effect of Taraxacum officinale extracts.** MCF-7/AZ breast cancer cells, pretreated with ET-18-OMe (15), and LNCaP C4-2B prostate cancer cells are invasive into collagen type I layer. DRE blocked the induced invasion of MCF-7/AZ cells into collagen type I gel layer, while DFE and DLE had no effect on the invasive behavior of...
MCF-7/AZ cells (Fig. 2A). Invasion of LNCaP C4-2B cells into collagen type I gel could be blocked by treatment with DLE and not by DFE and DRE treatment. Further evidence for the anti-invasive effect of DRE and DLE is shown in Fig. 2B, demonstrating decreased activity levels of both MMP-2 and MMP-9. These observations were found to be mediated through the rapid (5-10 min) inhibition of the elevated activity levels of both FAK and src (Fig. 2C). No changes in activity levels of MMPs and FAK and src were detected for the extracts that did not influence invasion (data not shown).

Discussion

Medicinal herbs and plants continue to play a significant role in drug discovery and development, particularly in cancer research. The overwhelming contribution of natural products to the expansion of the chemotherapeutic arsenal...
is evidenced by the fact that 50% of all the anticancer drugs approved worldwide between 1940 and 2006 were either natural products or natural product derived (18). Many of these novel anticancer agents have a history of use in traditional medicine, notably in TCM. The most prominent among them are: i) camptothecin, isolated from the Chinese ‘happy tree’ Camptotheca acuminata (19); ii) podophyllotoxin from Podophyllum peltatum, originally used by Native Americans but also found in large amounts in Podophyllum emodi var. Chinense (20); and iii) paclitaxel initially obtained from Taxus brevifolia and present in high quantities in Taxus chinensis (21).

Although plants of the genus Taraxacum, including Taraxacum officinale, are often used in TCM for the treatment of cancer and there is anecdotal information on the anticancer activity of TO, scientific evidence to support this effect, is lacking. In the present study, we showed that the aqueous DLE significantly reduced the growth of MCF-7/AZ breast cancer cells, in a dose-dependent manner that was not due to its direct cytotoxicity, as analyzed by the MTT assay. DLE did not influence the growth of LNCaP C4-2B prostate cancer cells neither did similarly prepared aqueous extracts from the root and the flower. Additionally, the effect on cell proliferation could be ascribed to the inhibition of ERK activity, with ERK being a major determinant in the MAPK-pathway involved in cell survival, differentiation and cell growth (22). Inhibition of tumor cell proliferation by Taraxacum extracts has been reported earlier and was found to be due to triterpenoids and sesquiterpenes (5,6). Since these compounds are also present in the roots, and no effect of DRE was observed on the growth of either cell line, our results suggest that the inhibitory effect may result from phenolic compounds as dandelion leaves are characterized by higher polyphenolic acids and flavonoid contents (23). This is further supported by several studies that describe the effect of the latter compounds, in particular polyphenols on cancer cell proliferation (24).

The invasive behavior of tumor cells is another important phenomenon affected by the different TO extracts. As demonstrated by the collagen type I invasion assay, DLE inhibits the invasiveness of LNCaP C4-2B cells, while no effect could be observed on the invasion of MCF-7/AZ cells. In addition, we found that extracts of the dandelion root inhibited the invasive behavior of MCF-7/AZ, but not of LNCaP C4-2B cancer cells. This effect was clearly observed after 24 h and was not due to reduced growth of the particular cancer cells. The inhibitory effect of DLE and DRE on the invasiveness of LNCaP C4-2B and MCF-7/AZ cells, respectively, was further evidenced by zymographic analysis, revealing that DLE and DRE inhibit the gelatinolytic activity of MMP-2 and MMP-9, given that the enzymatic activities of these matrix metalloproteinases correlate with tumorigenicity and metastatic ability of tumor cells (25). Moreover, signal transducers FAK and src, contribute to the secretion of matrix metalloproteinases 2 and 9, and activity levels are found elevated in invasive MCF-7/AZ cells (26) and in LNCaP C4-2B cells (unpublished data). As a result, we found that the anti-invasive effects of DLE and DRE are mediated through the inhibition of FAK and src activity. To the best of our knowledge this is the first study reporting the anti-invasive activity of extracts of Taraxacum species and especially of TO. The exact reason for the reduction in invasion can not be explained at present, as many of the known components in the roots and leaves can contribute to the observed effect (27-29) and this also depends on the composition of the extracts of the studied species.

Further fractionation and isolation of the active ingredients in the individual extracts will be crucial for elucidating: i) the anti-proliferative effect of DLE on MCF-7/AZ cells as well as ii) the differential effect of DRE and DLE on the invasive behavior of MCF-7/AZ and LNCaP C4-2B cells.

Medicinal plants, originating from or related to TCM, play an important role in the treatment of cancer and represent a valuable source for the discovery of small molecule inhibitors targeting signal transduction proteins, e.g. kinases, that modulate proliferation and invasion of cancer cells. Examples of such components derived from TCM include indirubin, inhibiting cyclin-dependent kinases, and emodin influencing a variety of signaling molecules among them, FAK and the PI3K-Cdc42/Rac1 pathway (30-32).

In summary, our data demonstrate that the aqueous extracts of different parts of TO inhibit cell proliferation and invasion and illustrate the importance of validating the use of traditional medicinal plants and herbs in therapy. Furthermore, these results indicate that DLE and DRE contain active compounds, which may be used in the development of new agents to combat cancer.

Acknowledgements

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References


