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Taraxacum officinale extract shows antitumor effects on pediatric cancer cells and enhance mistletoe therapy

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ABSTRACT

Objectives: While *Taraxacum officinale* (dandelion) extracts showed antitumor effects on adult cancer cells, effects on pediatric tumor cells as a single agent or in combination with mistletoe extracts are hitherto unknown.
Material and methods: The anti-proliferative effects of an aqueous fermented *Taraxacum officinale* extract (*Taraxacum*) on a pediatric cancer cell line panel were assessed by cell viability assays (MTT). In two neuroblastoma cell lines, SH-SY5Y and Kelly, the effects on cell cycle distribution (PI staining), mitochondrial integrity (MitoTracker staining), invasion (Boyden chamber assay) and migration (Scratch-assay) as well as the synergistic effects of the co-treatment of *Taraxacum* and mistletoe preparations (*Iscucin® Tiliae* or *Iscucin® Pini)* were investigated.
Results: All tested cancer cell lines were more susceptible to *Taraxacum* than the normal human fibroblast cell line, NHDF-C. In neuroblastoma cell lines *Taraxacum* caused apoptosis and loss of mitochondrial integrity as well as an inhibition of invasion and migration. The simultaneous therapy of *Taraxacum* and the mistletoe extracts revealed synergistic effects.
Conclusion: This preclinical data support the use of *Taraxacum* as a potential adjuvant application in pediatric oncology.

1. Introduction

Although there has been improvement in the diagnosis and treatment of childhood cancers in recent years, some tumors are still associated with a poor prognosis. For example, the 5-year survival rate in high-risk patients with *MYCN* amplification and stage 4 neuroblastoma (NB) is only 30–40%.[1,2] Tumors appear with a heterogeneous clinical behavior, ranging from spontaneous remission to aggressive and invasive tumor dissemination. Prognostic factors include stage and age of diagnosis as well as molecular features, such as *MYCN* amplification or *ALK* mutation.[3,4] Efforts to further improve the chemotherapeutic regimens of standard and high-risk therapy are ongoing. Progress in identifying the underlying genetic basis of high-risk relapsing NB suggests that improved survival in high-risk subgroups of NB will rely on novel treatment modalities that will lead to synergism with standard chemotherapy.

Parents of children suffering from cancer often choose alternative therapies adjunctively to aggressive chemotherapy to support conventional treatments or to attenuate side effects.[5] Medicinal herbs and plants have been used since ancient times for curing diseases.[6] Even today they play a significant role in drug discovery and development, particularly in cancer research.[7] Many cytostatic drugs are originally derived or isolated from plants (e.g. taxane from *Taxus baccata*) and screening plant specimens provides an exciting potential for detecting anti-tumor agents. A common treatment approach is the use of mistletoe (*Viscum album, Santalaceae*).[8,9] Historically, mistletoe was already administered in ancient medicine to cure diverse diseases and was revered as a holy plant by the Celts. However, only in the 1920s mistletoe has been introduced to oncology by Rudolph Steiner, founder of Anthroposophy.[10] Since then, mistletoe therapies are being administered with increasing frequency and popularity even in pediatric cancer treatment.[11,12] The most striking features of *Viscum album* extract are the cytotoxic and growth inhibitory effects on a variety of tumor cell lines and lymphocytes *in vitro*.[13,14] The cytotoxic effects of *Viscum album* extracts are mainly caused by the apoptosis-inducing mistletoe lectins, while the viscotoxins induce necrotic cell death.[15–18] *Viscum album* extracts are also known for their immunomodulatory activity. *In vitro* and *in vivo*, they activate monocytes/macrophages,

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granulocytes, natural killer cells (NK cells), T cells (especially T-helper cells) and induce various cytokines.[9,18]

Taraxacum officinale, the scientific name for common dandelion, is a widespread flowering herbaceous perennial plant, belonging to the *Asteracea* family. It is used for nutritional, medicinal and pharmaceutical purposes, promoting diuresis[19] and cure of gastrointestinal disorders as a phytotherapeutic agent (recommendations of the European Scientific Cooperative on Phytotherapie (ESCOP) and German Comission E). In anthroposophic medicine, *Taraxacum* preparations are used as bitters (*Amara* drops), in cases of digestive weakness with flatulence (*Aquilinum comp*. Globuli) and disturbed liver function (*Taraxacum Stanno cultum*).[20] From an anthroposophical viewpoint, *Taraxacum* qualifies for therapeutic applications in oncology from observations of its overflowing heat ether.[21]

Taraxacum officinale plant extract contains a wide range of pharmacologically active ingredients such as sesquiterpene lactones, phenylpropanoids, triterpenoids/sterols and flavonoids including luteolin 7-glycoside.[22–24] Recent studies suggested dandelion as a novel anticancer candidate. Aqueous dandelion root extract (DRE) showed antineoplastic effects on aggressive and resistant human chronic myelomonocytic leukemia (CML) cells, MV-4-11, HL-60 and U-937.[25] The observed effect was tumor cell specific, as non-cancerous peripheral blood mononuclear cells (ncPBMCs) and normal human fibroblasts remained unaffected. Furthermore, a loss of mitochondrial membrane potential was detected 24 h after treatment in MV-4-11. An activation of caspase-8 was also found in Jurkat, human T cell leukemia cells. In addition, earlier observations revealed that a dose of 2.5 mg/ml (DRE) was sufficient to induce apoptosis in the A375 human melanoma cell line, whereas G361 melanoma cells were almost unsusceptible to DRE treatment.[26] Sigstedt et al. (2008) examined the effects of aqueous extracts of different dried parts of *Taraxacum officinale* and found reduction of cell growth in the MCF-7/AZ breast cancer cell line, while LNCaP C4–2 B prostate cancer cells were unaffected. Dandelion leaf extract and DRE were both found to decrease enzymatic activity levels of matrix metalloproteinases, MMP-2 and MMP-9, as well as of the FAK-Src signaling pathway.[27] Thus, preclinical data suggest anti-proliferative effects of dandelion extracts on a variety of cancer cell lines.

The aim of the present study was to provide first preclinical data for general cytotoxicity of dandelion on a panel of pediatric tumor cell lines. Furthermore, the impact on cell viability, apoptosis, mitochondrial integrity, invasiveness and migration on two NB cell lines was investigated. Finally, combinatory effects of *Taraxacum* and mistletoe extracts were assessed.

2. Materials and methods

2.1. Taraxacum and mistletoe preparations

Taraxacum officinale extract, Taraxacum e planta tota ferm 34c (subsequently referred to as Taraxacum) and mistletoe preparations (*Iscucin® Tiliae* and *Iscucin® Pini*) were provided by *WALA Heilmittel GmbH* (Bad Boll/Eckwälden, Germany). Taraxacum e planta tota ferm was manufactured according to an official production method 34c laid down in the German Homeopathic Pharmacopoeia (GHP) and is commercially available as dilutions thereof (e.g. Taraxacum e planta tota D3). The extract is produced from comminuted fresh flowering plants, which undergo a lactic acid fermentation process during 7 days followed by a maturation period of six months at 15 °C.

Iscucin® Tiliae and *Iscucin® Pini* are aqueous mistletoe extracts according to GHP production method 38, from lime tree and pine.[28,29] and thus differ in their spectra of ingredients including the mistletoe lectins and viscotoxins. For calculating dose dilutions, the concentration of *Taraxacum* extract was determined as 1 g/ml. Iscucin preparations were diluted and potentized by *Wala Heilmittel GmbH* until strength of H, which was assumed to exhibit a concentration of 50 mg/ml.

2.2. Cell lines

A panel of 18 cancer cell lines was analyzed to investigate sensitivity to *Taraxacum* treatment: MV4-11 (acute myeloid leukemia; AML), RAMOS, NAMALWA, RAJI (Burkitt's lymphoma), TC-71, CADO-ES1, RD-ES (Ewińgs sarcoma), Daoy, ONS-76 (medulloblastoma), Kelly, SH-SY5Y, LAN-1, NLF, NB69, SK-N-AS (neuroblastoma), Saos-2 (osteosarcoma), G-401 (renal rhabdoid tumor), A-204 (rhabdomyosarcoma). Additionally, susceptibility of the human fibroblast cell line, NHDF-C, was tested. All cells were maintained in RPMI-1640 medium (Invitrogen, Waltham, MA, USA) or DMEM medium (Invitrogen) supplemented with 10% FCS (fetal bovine serum; PAA Laboratories GmbH, Cölbe, Germany), 100 U/ml penicillin, 100 µg/ml streptomycin (Invitrogen) and 3.5 µg/ml amphotericin B (Invitrogen) in culture flasks at 37 °C with 5% CO_2 in a humidified incubator.

2.3. Cell viability assay

Cell viability and IC50 values were determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assay.[30] Cells were seeded onto 96-well plates in octuplicates. After 24 h, they were treated with serial dilutions of *Taraxacum* and cell viability was assessed after another 72 h by addition of 50 µl of MTT solution (5 mg/ml in PBS; Roth GmbH, Karlsruhe, Germany) to each well. Plates were incubated for further 4 h at 37 °C, 5% CO_2 protected from light. After removal of media, blue formazan crystals were dissolved in 100 µl MTT solubilization solution (SDS 10%, 0.6% glacial acetic acid in dimethyl sulfoxide (DMSO)). The absorbance was measured at 570 nm with a reference wavelength of 690 nm after shaking plates for 30 min. Values were normalized to untreated controls.

2.4. xCELLigence proliferation time course analysis

Cell proliferation was monitored by the xCELLigence technology (ACEA Bioscience, Inc., San Diego, USA), according to the manufacturer's recommendations. Briefly, the device enables a continuous real-time monitoring of adhesion, proliferation and spreading of cells by detection of variations in electrical impedance that are transformed to a dimensionless factor referred to as "cell index".

Treatment with serial *Taraxacum* dilutions was conducted 24 h after seeding 2000 SH-SY5Y and Kelly cells per well on E-plates, equipped with electrodes integrated in its base. The cell index was normalized to the time point of *Taraxacum* addition. Growth curves were obtained and compared by RTCA software (ACEA Bioscience). Each dilution was tested fourfold.

2.5. Migration assay

Cell migration was assessed by "wound healing" experiments using confluent monolayers of cells in 6-well microtiter plates. The tip of a micropipette was then used to create a linear scratch resulting in two cell fronts, which were ~2 mm apart from each other as described earlier.[31] NB cells (Kelly and SH-SY5Y) were exposed to two *Taraxacum* concentrations (10 mg/ml and 100 mg/ml). Cell migration was judged by photographs taken immediately after scratching and at 24 h and 48 h after scratching using a digital camera and ImageJ software.[32] The test was conducted in four independent experiments.

2.6. Boyden chamber invasion assay

The motility of neuroblastoma cell lines, SH-SY5Y and Kelly, was quantified using the BD BioCoat Growth Factor Reduced Matrigel Invasion Boyden chamber assay (BD Biosciences, Bedford, MA). Cells were seeded in the upper inserts, simultaneously treated with *Taraxacum* dilutions and enabled to migrate along an FCS concentration gradient (5–15%). After 48 h, cells on the bottom of the inserts were