In vitro Anticancer Activity of Novel Bioactive Agents in Chemoresistant Tumors

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No effective or specific antineoplastic agent is available for the treatment of neuroendocrine tumors. Presently, the only effective therapy is restricted to radical surgery while chemotherapy has only palliative functions. We have established neuroendocrine tumor cell lines derived from a medullary thyroid carcinoma (MTC-SK)[1], and from a midgut carcinoid (KRJ-I)[2] as models for the analysis of new potential chemotherapeutic drugs.

Extracts and fractions were prepared from *Trailliaedoxa gracilis* WW Sm. & Forrest, a monotypical endemic Rubiaceae located in the province of Yunnan, China, and studied with MTC-SK and KRJ-I. Studies with Casy-1 Cell Counter & Analyzer ^{TM} (Schärfe System) and the WST-1 cell proliferation assay (Roche Diagnostics) showed a dose-dependent inhibition of cell growth of the tumor cells, while normal control cells were not impaired. Treated cells were viewed by a FEI Technai12 equipped with a Gatan CCD Camera Bioscan. The analysis of these cells focused on the nucleus and its relation to the cell. Cell membrane and other organelles were also examined to exclude the possibility of a necrotic damage caused by the dichloromethane fraction. The amount of treated surviving cells was compared with the living cells in the control (DMSO). The results substantiate the suspicion of apoptosis leading to other tests to analyse the fraction and to verify the initiation of apoptosis.

Caspase tests showed an activation of initiator- and effector-caspases in MTC-SK and KRJ-I cells treated with the dichloromethane fraction of *Trailliaedoxa gracilis*. Positive control was 5 µM Camptothecin, negative control was DMSO. Caspase 8 activity was enhanced early. The fraction containing ursolic acid might suppress NF-κB activation[3] or inhibit NEMO activity[4]. *In vivo*-experiments with heterotransplanted immunodeficient SCID mice showed a clear tendency towards tumor inhibition.

HPLC and NMR Analysis showed the presence of ursolic acid, and betulin and sitosterol[5]. Ursolic acid, a pentacyclic triterpene acid, was described for a supression of NF-κB activation induced by various carcinogens[3]. The action of ursolic acid on neuroendocrine tumor cells has never been studied before. Ursolic acid might be a new drug for chemotherapy of the so far chemo-resistant neuroendocrine tumors.

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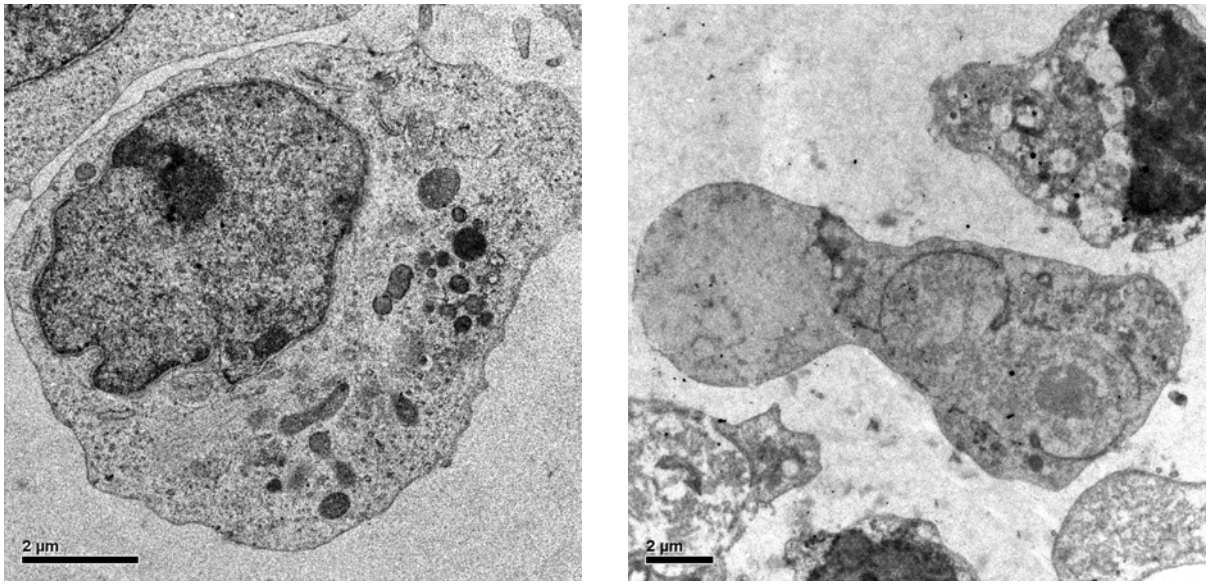

Figure 1. Left side: MTC-SK cells treated with 10µg/ml DMSO (negative control).
Right side: MTC-SK cells treated with 10 µg/ml dichloromethane fraction of *Trailliaedoxa gracilis* WW. Sm. & Forrest (TG-F28).

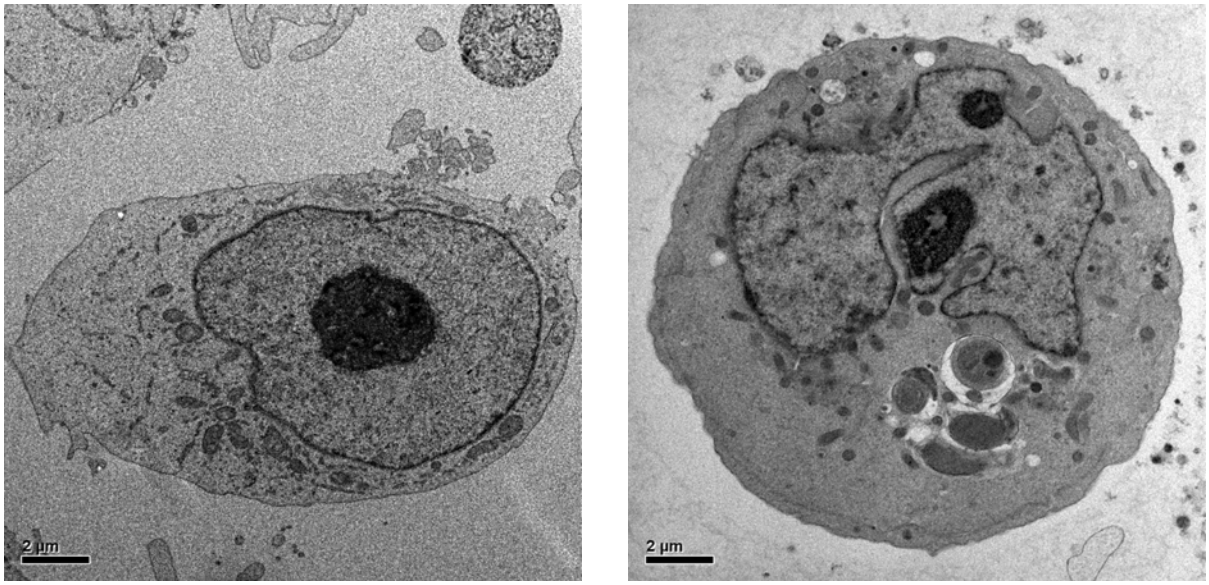

Figure 2. Left side: KRJ-I cells treated with 10µg/ml DMSO (negative control).
Right side: KRJ-I cells treated with 10 µg/ml dichloromethane fraction *Trailliaedoxa gracilis* WW. Sm. & Forrest (TG-F28).

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