

Comparison of chemosensitivity testing of CETCs and spheroids in cancer patients with solid tumors

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Abstract

Background: In vitro chemosensitivity testing of circulating epithelial tumor cells (CETCs) provides a real-time information about the sensitivity of the tumor cells present in the patient and correlates with treatment success. Nevertheless, a fraction of CETCs can survive after conventional chemotherapy and grow into distant metastasis. A subpopulation of CETCs with proliferation activity has the ability to form spheroids in suspension culture. Spheroids exhibit stem cell-like properties and may be responsible for chemo therapeutic resistance. Therefore, the aim of our study was the comparison of the efficacy of chemo therapeutics on CETCs and on spheroids originating from the same individuals.

Methods: The enumeration of CETCs collected from patients with solid tumors in clinical stage 1-4 were performed using the maintrac® method. Subsequently, viable CETCs were cultured in suspension culture system allowing for spheroid formation. To evaluate the cytotoxic effect, CETCs and spheroids in short time cultrues were exposed to different concentrations of anticancer drugs for different periods of time.

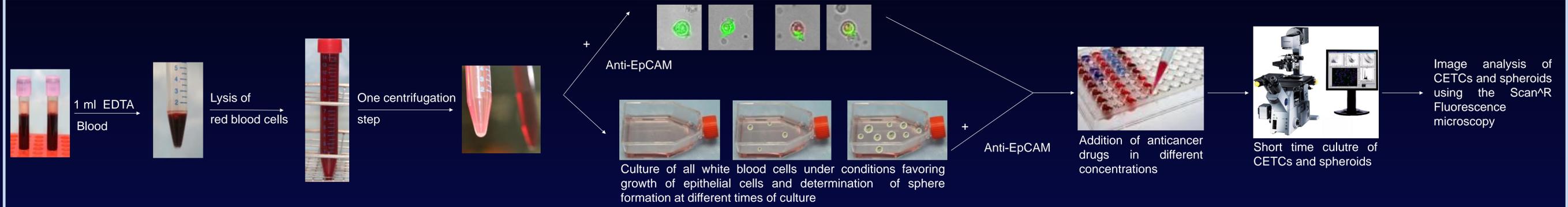
Results: The response to chemotherapeutics was different in CETCs and spheroids. In contrast to CETCs, spheroids from the same patients were significantly more chemoresistant. Whereas active drugs led to membrane permeability in single CETCs with subsequent staining of the nuclei with propidium iodide, the same drugs led to disintegration of tumorspheres with destruction of part of the cells but often part of the cells in the spheres was able to survive. Epirubicin and, interestingly, and especially salinomycin, a polyether ionophore antibiotic isolated from *Streptomyces albus*, showed the best effects. Docetaxel, cyclophosphamide and 5-fluorouracil showed almost no cytotoxic effects onto the cells in the spheres.

Conclusion: Our results show, for the first time that stem cells circulating in peripheral blood, capable of forming spheroids are way more resistant to anticancer drugs than the remnant circulating tumor cells. We, furthermore, demonstrate that salinomycin efficiently destroys spheroids cultured from CETCs, strengthening its role as a promising anti-cancer therapeutic.

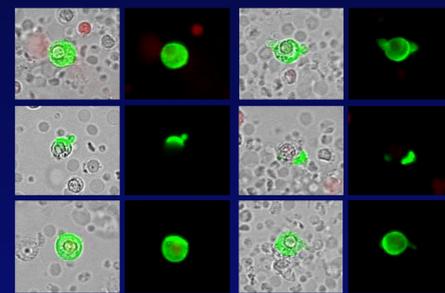
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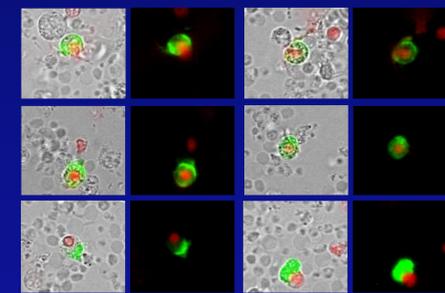
Methods



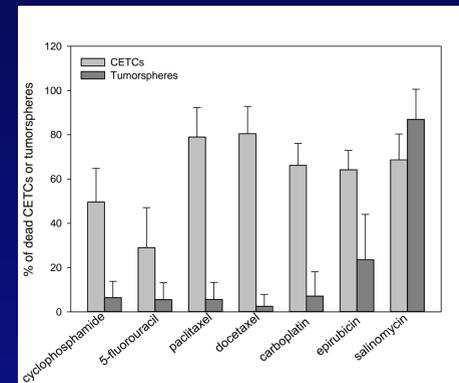
Results



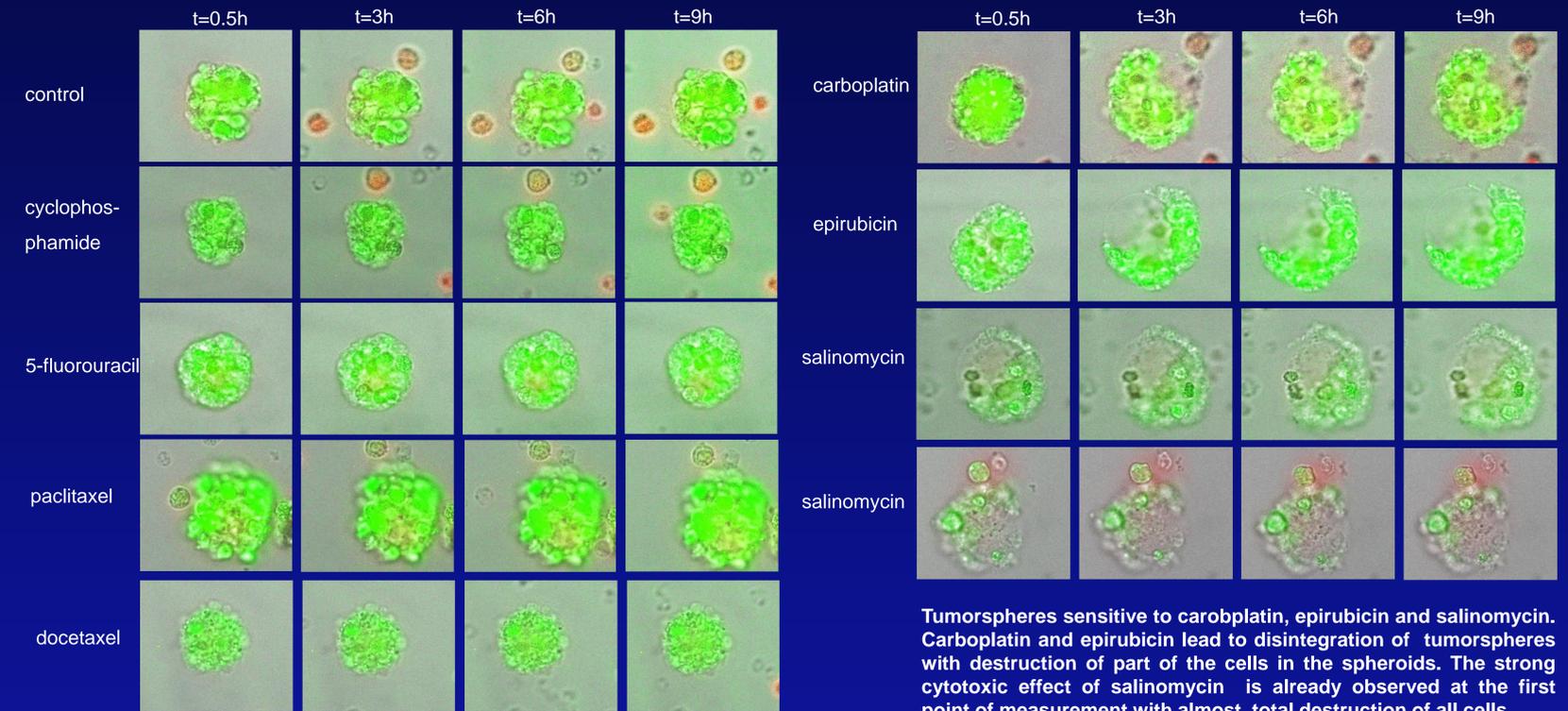
Remaining live CETCs after cytotoxic drug treatment. These cells have an intact morphology with a well preserved membrane without PI staining in the nucleus.



Dead CETCs after short time culture with cytotoxic drugs. Cells show a positive PI staining because of loss of membrane integrity.



Comparison of the cytotoxic effect of different drugs on CETCs and tumorspheres. Spheroids are more resistant than CETCs from the same patient, which confirmed our hypothesis that a small fraction of CETCs has stem cell properties. Most of the tested anticancer therapeutics show statistically significant higher activity on CETCs. Interestingly, salinomycin treatment significantly reduces the number of viable tumorspheres more than of the CETCs.



Examples of tumorspheres with chemoresistance to cyclophosphamide, 5-fluorouracil, paclitaxel and docetaxel. Tumorspheres remain alive during short time culture (0-9h).

Tumorspheres sensitive to carboplatin, epirubicin and salinomycin. Carboplatin and epirubicin lead to disintegration of tumorspheres with destruction of part of the cells in the spheroids. The strong cytotoxic effect of salinomycin is already observed at the first point of measurement with almost total destruction of all cells.