In vitro chemosensitivity testing of mammospheres cultured from circulating epithelial tumor cells (CETCs) of breast cancer patients: Comparison to chemosensitivity of total CETCs.

Abstract:

**Background:** In vitro chemosensitivity testing of circulating epithelial tumor cells (CETCs) provides real-time information about the sensitivity of the tumor cells present in the patient. Nevertheless, a fraction of CETCs can survive after conventional chemotherapy and grow into distant metastases. This may be a subpopulation of CETCs with proliferative activity which has the ability to form floating spheres in suspension culture. Spheroids exhibit stem cell-like properties and may be responsible for chemotherapy resistance. Therefore, the aim of our study was to determine the efficacy of chemotherapeutics on spheroids cultured from CETCs. **Methods:** The enumeration of CETCs from patients with solid tumors in clinical stage I to IV was performed using the mamstrat method. Subsequently, CETCs in the context of the surrounding white blood cells were cultured in a suspension culture allowing for spheroid formation. To evaluate the cytotoxic effect of drugs on CETCs and spheroids we exposed them to anticancer drugs in short time culture in different concentrations and for different periods of time. **Results:** In contrast to CETCs, spheroids were significantly more chemotherapy resistant. Active drugs led to disintegration of tumor spheres. Interestingly, some cells in the spheres were able to survive. Epirubicin and especially salinomycin, a polyether ionophore antibiotic isolated from Streptomyces albus, showed high efficacy in a high proportion of cells. Furthermore, our data suggested that curcumin, a natural biologically active compound that is extracted from the plant Curcuma longa, is a promising agent for cancer treatment. Docetaxel, cyclophosphamide, and 5-fluorouracil showed lower cytotoxic effects onto the cells in the spheres. **Conclusions:** 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 and curcumin efficiently destroy spheroids cultured from CETCs, strengthening their role as promising anticancer therapeutics.