Local tumor-cell stress induced by modulated electro-hyperthermia could lead to an abscopal effect by immune-promotion in C26 mouse colorectal carcinoma allografts

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Objective
Malignant tissues have elevated glycolytic activity (Warburg-effect) which causes higher lactic acid and ion content in the extracellular space, thus selective energy absorption can be applied by specific electric fields. Modulated electro-hyperthermia (mEHT, tradename: oncothermia) is a non-invasive complementary to chemo- and radiotherapy, which uses 13.56 MHz amplitude modulated field to induce cell stress (at 42°C) and damage. We showed that mEHT caused significant caspase-independent apoptosis and damage associated molecular pattern (DAMP) signal sequence in HT29 colorectal cancer xenografts of immunocompromised mice. Here we tested the mEHT following potential immune-response and tumor-damage in a mice allograft tumor model using immunocompetent animals.

Methods
Both femoral regions of Balb/C mice were subcutaneously inoculated with C26 colorectal cancer allografts were into. Right side tumors were treated with ~42°C mEHT for 30 minutes. The expression of heat shock, growth-, damage signaling and immune response associated proteins was tested in situ immunohistochemistry.

Results
mEHT treatment induced significant and progressive tumor damage in treated right-side tumors. Significant increase of cleaved/activated caspase-3 levels indicated caspase-dependent apoptosis, which was proved by the elevated cytochrome-c release from the mitochondria and the significant increase in TUNEL positive tumor cell nuclei as well. There were no such members of the intrinsic programmed cell death pathway as the translocation of apoptosis-inducing factor (AIF) from the mitochondria into cell nuclei, or displacement of Bcl-2-associated X protein (Bax) from cytosol to mitochondria. Significant release of hsp70, HMGB1 and calreticulin which are known participants of DAMP signaling was also showed in mEHT treated tumors. Furthermore the number of S100+ dendritic cells and CD3+ T cells was significantly increased in the treated tumors, while the number of FoxP3+ regulatory T-cells remained unchanged. In addition, mEHT combined with the i.p. administration of a CD8+ T-cell promoting chlorogenic-acid rich herbal seemed to initiate a significant tumor destruction in the untreated distant tumor site too.
**Conclusion**

The C26 colorectal adenocarcinoma allografts have high proliferation index and lead to cancer cachexia in mice, which partly due to the impaired immune-response. In this study, a single shot mEHT treatment could induce a primary caspase-dependent programmed cell death and the release of stress associated DAMP signals. These were followed by a progressive accumulation of antigen presenting dendritic cells and CD3+ T-cells referring to an immunogenic cell death (ICD) mechanism, which could be extended to systemic anti-tumor response by a T-cell promoting agent.

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Malignant tissues have elevated glycolytic activity (Warburg effect) which causes higher lactic acid and ion content in the extracellular space, thus selective energy absorption can be applied by specific electric fields. Modulated electro-hyperthermia (mEHT, oncothermia) is a non-invasive complementary to chemotherapeutic which uses 13.56 MHz amplitude modulated field to induce cell stress (at 42°C) and damage. We showed that mEHT caused significant caspase-dependent apoptosis and damage associated molecular pattern (DAMP) signal sequence in HT29 colorectal cancer xenografts of immunocompromised mice. Here we tested the mEHT following potential immune-response and tumor-damage in a mice allograft tumor model using immunocompetent animals.

C26 mouse colorectal carcinoma cell line was inoculated into both femoral regions of mice. mEHT was applied for 30 minutes on the right side and the tumor-core temperature was kept below 42°C.

mEHT caused significant and progressive tumor destruction, which was measured by the ratio of damaged tissue to living area.

12 and 24 h after mEHT treatment mitochondrial Bax and cytoplasmic cytochrome c relocalization and elevated cleaved caspase-8 and -3 levels indicated caspase mediated apoptosis, which was proved by nuclear DNA fragmentation (detected by TUNEL).

Conclusion: The progressive tumor destruction after a single shot mEHT probable caused by an ICD mechanism, which can be extended to systemic response by immune-promotion.

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