Metformin Selectively Impairs Canine Mammary Cancer Stem Cell Survival In Vitro And In Vivo

F. Barbieri[1,2], S. Thellung[1,2], R. Wurth[1,] A. Daga[3], A. Bajetto[1], A. Ratto[4], A. Ferrari[4], and T. Florio[1,2]
[1]Section of Pharmacology – Dept. of Internal Medicine (DIMI); University of Genova, Italy
[2]Center of Excellence for Biomedical Research (CEBR); University of Genova, Italy
[3]Gene Transfer Lab; IRCSS AOU San Martino-IST; Genova, Italy
[4]Centro di Referenza per l'Oncologia Veterinaria e Comparata (CEROVEC), IZS-PLV; Genova, Italy

Cancer stem cell (CSC) concept proposes that a subpopulation of tumor-initiating cells within tumors drives development, progression and relapse of several malignancies, including breast cancer. CSCs display high chemo- and radio- resistance responsible of therapy failure, tumor relapse and metastasization, through their exclusive ability to self-renew and repopulate the tumor. Thus, the identification of new therapeutic approaches targeting CSC survival represents a great challenge to improve antitumor efficacy. Comparative oncology studies report the isolation of CSCs also in naturally occurring cancer of companion animals. Spontaneous cancer models, maintaining individual tumor complexity and patient heterogeneity, can be a valuable tool for translational research in novel cancer drugs. Particularly, dogs develop mammary carcinomas that closely mimic the biology of same form of cancer in humans. Metformin, an oral hypoglycemic drug, displays potential anticancer effects, specifically targeting CSCs in human breast cancer. As pre-clinical research on canine tumors can provide valuable information that will lead to improved understanding and treatment of human and animal cancers, in the present study we evaluated the effects of metformin on CSCs from canine mammary carcinomas (CMCs) in vitro and in vivo.

Primary cultures were derived from surgical specimens of CMCs and maintained in stem cell-permissive medium containing EGF and bFGF. The expression of CD44, a human breast CSC marker, was analyzed by immunohistochemistry on tissues and by immunofluorescence on isolated cells to detect the presence of CSC-enriched mammary cultures. According to a consensus definition, we verified that cultures possess the capacity to self-renew, proliferate in vitro, differentiate in fetal calf serum-containing medium and recapitulate the original tumor in NOD/SCID mice xenotransplantation, thus representing a reliable model to identify drugs selectively targeting this cell population. Dose-response curves (1-100 mM) carried out on 12 cultures maintained in CSC-enriching conditions and their differentiated counterpart showed that metformin preferentially inhibits CSC survival, with a mean IC_{50} value of 30 mM. By contrast, CMC CSC proliferation was slightly affected by the treatment with doxorubicin, that significantly impairs differentiated CMC cell viability (IC_{50}=0.41 μM). Interestingly, as P-glycoprotein transporter (Pgp) confers CSC the ability to resist to cytotoxic drugs by actively pumping them out of cells, we were able to increase the intracellular uptake of doxorubicin in CSCs and revert resistance by using the Pgp inhibitor verapamil, obtaining comparable IC_{50} value (0.44 μM). The antiproliferative effects of metformin were confirmed in vivo, by injecting TICs and corresponding differentiated cells in the fat pad of NOD/SCID mice, then treated with metformin 360 mg/kg/die dissolved in drinking water, for 6 months. The plasma concentration of metformin was measured (6.90 mg/ml) to evaluate pharmacokinetics and actual drug intake. Metformin treatment of tumor bearing mice resulted in a significant reduction of the tumor mass (60% *vs.* untreated controls). Immunohistochemistry on explanted tumors showed a reduced number of CD44-positive cells in metformin-treated tissues and cells derived from these tumors showed a reduced clonogenic potential as compared to those isolated from untreated xenografts, suggesting the selectivity of metformin antiproliferative effects toward CMC CSCs.

Effective treatment of cancer requires the eradication of all cell types within a cancer, namely, CSCs, more differentiated progenitors and the bulk tumor cell population that might be achieved by combining CSC targeting agents with conventional cytotoxic drugs, as metformin and doxorubicin. These data show that CMC cultures enriched in CSCs are a suitable model for testing drugs aimed at eradicating this cell subpopulation.