MRI/ MRS on Leukemia Development in MLL-AF9 Transgenic Mice

D. DeRyckere[1], M. E. Macy[1], K. M. Hasebroock[2], L. A. Gardner[3], P. Jedlicka[4], E. L. Bradshaw-Pierce[2], A. L. Merz[2], L. Gore[1], and N. J. Serkova[2]

[1]Medical Oncology, University of Colorado Health Sci, Aurora, CO, United States, [2]Anesthesiology, University of Colorado Health Sci, Aurora, CO, United States, [3]Pediatrics, University of Colorado Health Sci, Aurora, CO, United States, [4]Pathology, University of Colorado Health Sci, Aurora, CO, United States

Introduction

Standard methods to assess tumor progression and treatment response for leukemia patients rely on repeat sampling of bone marrow. These methods evaluate overall disease status, but are not sensitive to early biochemical or cellular responses to therapies. Identification and validation of additional pharmacodynamic endpoints that are useful for accurate prediction of therapeutic efficacy would allow for earlier assessment of clinical response and thereby facilitate the development of effective individualized treatment regimens. Ideally, these markers would be assessed using non-invasive methods. Toward this end, we used *in vivo* magnetic resonance imaging (MRI) and *ex vivo* magnetic resonance spectroscopy (MRS) to investigate changes in the bone marrow, spleen, and blood of leukemic MLL-AF9 transgenic (Tg) mice relative to wild-type littermates.

Methods

We have evaluated transgenic mice that express an MLL-AF9 fusion protein, which is a poor prognostic marker in children with acute leukemia. MLL-AF9 Tg mice develop leukemias with very high penetrance at a median age of approximately 5 months. These mice therefore serve as a model of high risk pediatric acute leukemia. T1-weighted MR images of leukemic MLL-AF9 Tg mice and age-matched wild-type control animals were obtained at 2 week intervals starting at 18 weeks of age using a Bruker 4.7 Tesla PharmaScan. Multi slice multi echo (MSME) T1-weighted sequence was used: FOV=4 cm; slice thickness 0.8 mm; coronal slice orientation; TR/TE = 720/ 11 ms; number of averages 4; flip angle 90 degree; total acquisition time 12 min 17 sec. The mice with overt leukemia received 250 mg/kg [1-13C] glucose i.v.; bone marrow, spleen, and blood were collected for metabolic ex vivo ^{1}H-, ^{13}C- and ^{31}P-MRS at a Bruler 500 MHz DRX spectrometer.

Results

MLL-AF9 Tg mice with leukemia exhibited a statistically significant 1.5-fold increase in bone marrow T1-weighted MRI signal intensity even prior to contrast administratiin n(Figure 1A). Increased signal intensity preceded development of leukemia and is therefore likely to be due, at least in part, to increased bone marrow cellularity, initially as a result of pre-neoplastic myeloproliferation and later as a result of marrow infiltration with leukemic blasts. After intravenous injection of 0.1 mmol/kg gadolinium-DTPA (Omniscan), a further increase in T1-signal intensity was observed in Tg mice (Figure 1B). No differences in microvessel density was found in the bone marrow of luekemic mice indicating that MRI changes are not due to increased angiogenesis. Leukemic MLL-AF9 Tg mice also exhibited statistically significant changes in metabolite levels in spleen, blood, and bone marrow. The Warburg effect, whereby cancer cells utilize aerobic glycolysis to meet their increased energy demands, was evident in all Tg tissues examined as indicated by increased glycolysis rates, increased glucose utilization, and increased levels of lactate and alanine, the end-products of glycolysis. Additional changes in metabolite levels were observed in the bone marrow and spleen of leukemic mice. Absolute levels of glutathione were increased. Glutathione reduces reactive oxygen species that are generated as a result of increased glycolysis and high levels of glutathione are associated with chemoresistance and poor prognosis in patients with acute leukemia as described previously. Increased glycine levels were also observed and may be associated with increased pyridine and DNA synthesis in leukemia cells. Decreased glutamate and glutamine levels may reflect a decrease in utilization of the mitochondrial Krebs cycle in tumor cells. Decreased levels of myo-inositol and taurine, which function as osmoregulators, may occur as a result of osmotic stress due to increased cellularity in leukemic organs. Levels of aromatic acids, lysine and arginine, and creatine and phosphocreatine were also decreased.

Conclusions

These results also demonstrate the feasibility of obtaining MR images of mouse bone marrow with sufficient resolution to compare wild-type and leukemic mice and suggest that increased T1-weighted MRI signal intensity may be useful as an indicator of bone marrow tumor burden. The data presented here also suggest novel metabolic targets for therapeutic intervention. Because metabolic changes often precede detectable changes in tumor burden, they may be particularly useful as early indicators of therapeutic efficacy and may thereby allow for more rapid determination of clinical response, decreased exposure to toxic therapies in resistant patients, and more expedient conversion to effective therapies. Because changes in glucose metabolism are a central feature of tumorigenesis and changes in glutathione levels have been associated with clinical outcome in patients with acute leukemia, these metabolites are of particular interest. Future studies will use MLL-AF9 Tg mice to investigate the roles of metabolic changes during *de novo* development of leukemia.

Figure 1: T1-weighted MRI on wild-type (WT) and transgenic (Tg) MLL-AF9 leukemic mice: (A) non-enhanced baseline; (B) changes after intravenous injection of 0.1 mmol/kg gadolinium-DTPA (Omniscan).