Metabolic shift in cancer cells
insights from an acute leukemia xenotransplantation model

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Abstract

Cancers evolve by a reiterative process of clonal expansion, genetic diversification and clonal selection within the adaptive landscapes of tissue ecosystems. This diversity entails also phenotypic features that include metabolic drifts in cancer cells. Strong microenvironmental selective forces (and therapeutic interventions) may therefore decimate cancer clones, and erode their habitats, and provide potent selective pressure for the expansion of resistant variants.

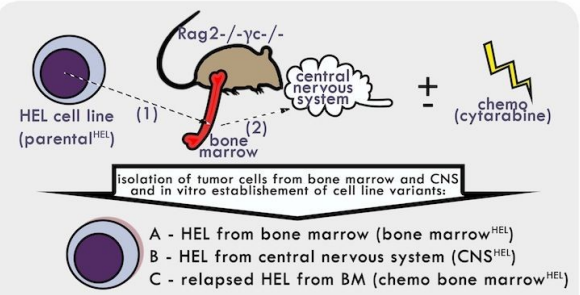
In an effort to establish a methodological framework for analysis of metabolites and metabolic pathways in this complex process, we have developed an experimental system to further dissect this process.

By selectively isolating tumor cells form the Central Nervous System and Bone Marrow, in an orthotopic xenograft model of acute myeloid leukemia (AML, HEL cell line, intra-tibia injection), we have derived several pairs of AML cell lines (CNS-AML and BM-AML) that originate from the same parental cell line but have colonized/invaded different organs.

Detailed in vivo serial transplantations assays, histological and electron microscopy analysis, ex-vivo 1H NMR spectroscopy, and RNA and protein expression profiles were used to compare the metabolic profile of these cells lines.

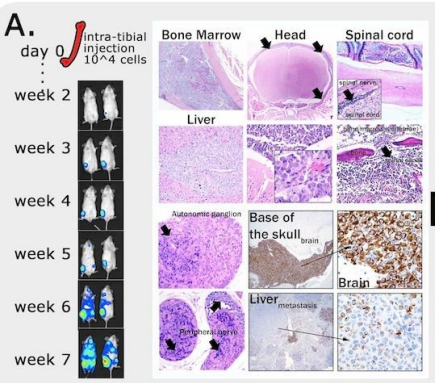
Our results show that both systemic and therapeutic pressures are associated with metabolic diversification and selection of tumor cell variants.

Methods



An orthotopic xenotransplantation model of human AML (hAML), based on injection of 10,000 HEL cells (pre-transduced with a lentiviral vector encoding luciferase and GFP) into the tibia (intra-bone marrow) of sublethally irradiated immunodeficient Rag2-/-γ chain-/- mice was conducted. Tumour progression was assessed by luminescence, flow cytometry and histology, and followed a highly reproducible pattern of leukaemia development. Leukemia cells were then isolated from bone marrow (femur) and central nervous system (brain) and were brought back to in vitro culture, to establish new cell line variants. We have also developed an in vivo chemotherapeutical treatment protocol aiming to induce complete remissions, followed by predictable recurrences of hAML in these IBM-xenotransplanted Rag2-/-γchain-/- mice. Briefly, leukaemic mice (>0.1% GFP+ hAML cells among the mononuclear cell fraction of blood) were treated with two cycles of 5 constitutive daily injections of 100 mg/kg cytarabine (AraC).

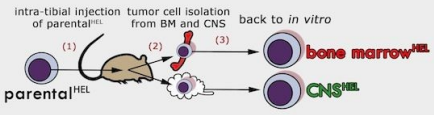
1. Intra-tibial xenotransplantation model

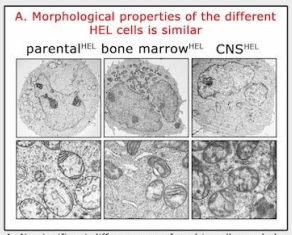


A. Over time, hAML cells spread from the bone marrow to the blood and then on to secondary organs such as the spleen, liver, kidneys, lung and brain, leading to death at ~50 days post-injection. This model suitably reproduce hAML growth, spreading and associated mortality.

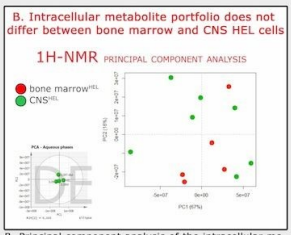
B. Treatment with cytarabine significantly decreased the total number of circulating hAML cells (as measured by quantitative flow cytometry) and resulted in dramatic decrease in leukaemia burden in the blood, bone marrow, spleen, liver and lung (as measured by both flow cytometry and histology) of treated animals, compared to untreated controls. Relapse occurs aprox. 3 weeks after treatment.

2. bone marrow HEL *Vs* CNS HEL cell lines: selective preasure of the MICROENVIRONMENT

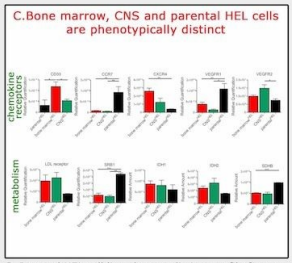




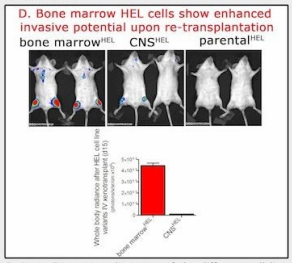
A. No significant difference was found in cell morphology, organelle distribution, and lipid droplets' density between the different cell line variants.



B. Principal component analysis of the intracellular metabolites in HEL cells, assessed by 1H-NMR, allows for discrimination of the parental HEL cells, but not in between bone marrow HEL and CNS HEL, as shown above. We can then conclude that the cell line variants from bone marrow and CNS are metabolically distinct from the parental cell line, and that these features are maintained in vitro.



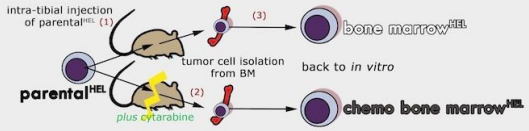
C. Parental HEL cell line shows a distinct profile for specific chemokine receptors and metabolism associated receptors/enzymes, when compared with bone marrow and CNS HEL cell line variants. Also, when comparing bone marrow and CNS, the first show increased expression of CD33 (stem cell marker) and CXCR4 (chemokine receptor associated with bone marrow homing and expansion of hAML cells).

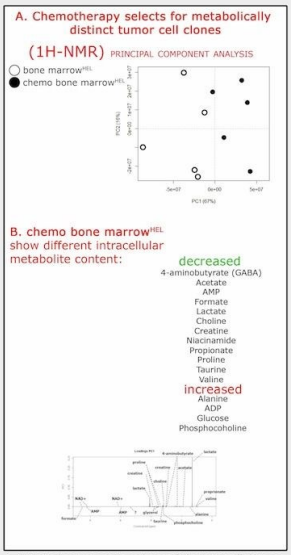


D. Upon IV re-transplantation of the different cell line variants, at day 15 post-transplant bone marrow HEL cells show enhanced expansion and colonization capacity, namely of the bone marrow and liver (as confirmed by histology) compared with CNS HEL cells. Both of these cell variants display increased aggressiveness, compared with the parental HEL cell line, which at day 15 only shows minimal residual disease, with no bone marrow or liver invasion. These results are support the molecular findings (panel C).

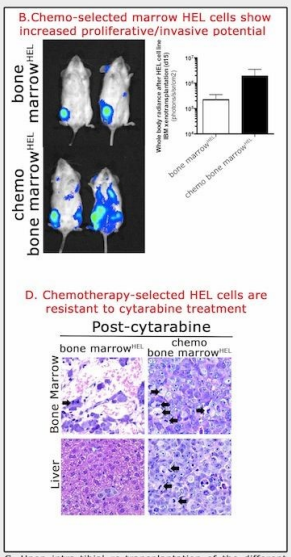
Systemic stimuli select for metabolically/phenotypically distinct tumor cells, with different invasive potential

3. bone marrow HEL *Vs* chemo bone marrow HEL cell lines - selective preasure of CHEMOTHERAPY





A. Principal component analysis of the intracellular metabolites, assessed by 1H-NMR, significantly discriminates bone marrow HEL cells from chemotherapy selected bone marrow HEL cells.
B. The latest show increased content of alanine, ADP, glucose and phosphocholine, while other metabolites are decreased.
We can then conclude that exposure to chemotherapy selects for metabolically distinct tumor cell clones, and that these features are maintained in vitro.



C. Upon intra-tibial re-transplantation of the different cell line variants, at day 15 post-transplant, bone marrow HEL cells previously exposed to chemotherapeutic agent (in vivo) show enhanced expansion and colonization capacity than "naive" bone marrow HEL cells.
D. These cells also show resistance to chemotherapy and mice xenotransplanted with chemo bone marrow cells fail to achieve clinical remission.

Chemotherapy is associated with selection of more invasive and metabolically/phenotypically distinct tumor clones

CONCLUSION

It has been recently shown by Ding et al., Nature 2012 (doi:10.1038/nature10738) that in humans, both ALL and AML share common features of clonal heterogeneity at presentation followed by dynamic clonal evolution at relapse, including the addition of new mutations that may be relevant for relapse pathogenesis. Clonal evolution is also known to occur after allogeneic transplantation (for example, loss of mismatched HLA alleles via a uniparental disomy mechanism), demonstrating that the type of therapy itself can affect clonal evolution at relapse; and taken together, these data demonstrate that AML cells routinely acquire a small number of additional mutations at relapse, and suggest that some of these mutations may contribute to clonal selection and chemotherapy resistance.

Here we show, in an orthotopic xenotransplant hAML model, that both the selective pressure of the systemic stimuli and chemotherapy imprint metabolic and other phenotypic alterations to cancer cells that contribute to chemotherapy resistance and enhanced malignancy/tumor progression. We further demonstrate that these changes are maintained after in vitro culture and re-transplantation. If these changes are associated with clonal selection or phenotypic drift is yet to be clarified. An in-depth analysis of the metabolome and RNA profile of these variant cell lines may therefore bring new insights to the clonal selection and chemotherapy resistance mechanisms in human AML.