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We have characterized on a prospective basis the behavior and functions of the $ALDH^{bright}$ sub-population of AML cells set at a cellular level. Previously we have shown that self-renewing, primitive HSC were found in the SDF (slow dividing fraction). Other authors have demonstrated that LSC behaved like their normal counterpart in this respect. In our study, the $ALDH^{bright}$ population contained two-fold more slow dividing cells than the $ALDH^{dim}$ population. Furthermore, cells capable of initiating leukemic LTC-IC were enriched in the $ALDH^{bright}$ subset and were practically absent from the $ALDH^{dim}$ subset. The leukemic LTC-IC frequency in the CD34+ cells was substantially lower than that in $ALDH^{bright}$ and $CD34+ALDH^{bright}$ cells. It remains unclear, whether $CD34+ALDH^{dim}$ LSC exist in relevant numbers. In our in vitro studies, $ALDH^{dim}$ cells were not able to generate secondary leukemic colonies.

In our NOD/SCID transplantation experiments, $ALDH^{dim}$ cells showed a leukemic engraftment during the observation time of about 60 days, albeit nowhere close to the range as $ALDH^{bright}$ cells. Given the significantly lower numbers of transplanted $ALDH^{bright}$ cells and the limited observation period, it may be that only $ALDH^{bright}$ cells are capable of sustained engraftment in the mouse model.

The adhesion chamber assay has also provided an appropriate technique for the analysis of the interaction between leukemia cells and the niche (Ran et al., 2009). Similar to their normal counterparts, LSC from the $ALDH^{bright}$ subpopulation showed a significantly higher adherence towards human MSC than the $ALDH^{dim}$ cells. We are concurrently investigating the relative roles of several

adhesion molecules for the interactions between human leukemia stem cells with the surrogate niche, i.e. MSC. Isolation of LSC candidates using CD34+ALDH^{bright} might provide a practical method for acquisition of an adequate amount of starting material for defining the cellular and molecular mechanisms of homing, adhesion, and the release of LSC from the niche. Such a method for the prospective separation of LSC from other leukemia blasts is also essential in the characterization of major molecular differences between normal HSC and LSC in their respective interactions, e.g. adhesion with the niche.

Most remarkably of all, our study has demonstrated the clinical significance of the presence of relatively high frequency of LSC candidates. For the first time, we have demonstrated in this study that higher percentages of LSC candidates as reflected by ALDH^{bright} cells are associated with significantly lower overall survival probability. This was true both in the cytogenetic high-risk as well as in the intermediate risk group. Other authors have also shown that ALDH activity was significantly associated with adverse prognostic features based on cytogenetic and molecular markers, but we have been able to demonstrate that ALDH positivity might also be another independent factor. Since over expression of ALDH results in chemotherapy resistance in human and murine HSC, the enzyme itself might play a functional role in this process and might serve as a potential therapeutic target for eliminating LSC.

In summary, assessment of the ALDH activity has provided a useful tool for the isolation of a distinct leukemia subpopulation with a high affinity to the surrogate niche, an exclusive capability of generating leukemia LTC-IC in vitro, a much more pronounced leukemia engraftment in the NOD/SCID mouse model, and, above all the frequency of these LSC candidates is associated with worse survival. These LSC candidates might be an appropriate starting material for further molecular and cellular characterization and for identifying targets for elimination of LSC for long term cure.

Above all, high percentages of these LSC candidates are associated with worse prognosis. Isolation of LSC candidates using ALDH might therefore provide a practical method for acquisition of an adequate starting material for defining the cellular and molecular mechanisms of homing, adhesion, and the release of LSC

from the niche and especially in identifying the essential molecular differences between normal HSC and LSC.

Furthermore, in conjunction with index sorting and single cell deposition, the functional and molecular characteristics of these LSC and normal HSC candidates will be determined and compared.