Proliferation and anti-apoptotic markers in myeloid malignancies

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Impact

The studies in this thesis contribute to our understanding of the role of cell division and cell death in myeloid malignancies. In MPN, the percentage of dividing (proliferating) cells was elevated, while the percentage of dividing cells of MDS was decreased. MDS/MPN showed features of MPN and MDS simultaneously. The percentage of dividing cells was even further decreased in AML, while the percentage of cells that are protected from cell death (anti-apoptosis) was increased in MDS and AML. The Ki-67 proliferation index in erythroid cells of the bone marrow was found to be of value for the diagnosis of MDS and integration of this parameter into the Ogata score can significantly increase its diagnostic sensitivity. Finally, it could be concluded that the Ki-67 proliferation index in erythroid cells of the bone marrow can predict transfusion-dependence in MDS patients with mild anemia. The optimal gating strategy for determining the Ki-67 proliferation index and Bcl-2 anti-apoptotic index was determined in order to warrant accurate conclusions and promote subsequent clinical implementation and standardization of these parameters.

Scientific impact

The evidence on the role of these cell biological processes in the pathogenesis, the transformation of MDS into AML and therapeutic response is contradictory and originates primarily from the 1990s. The limited number of parameters that could be simultaneously analyzed with (2- to 4-color) flow cytometry at that time prevented the implementation of these biomarkers for diagnosis, prognosis and/or therapy response, although they are proven to be of high potential for clinical practice of these malignancies. To this date, routine clinical practice primarily focused on identification and characterization of malignant stem- and progenitor cells by means flow cytometric analyses of CD marker expression profiles, while changes in cell biological processes are fundamental in the oncogenesis and neoplastic progression. No complete analysis of cell biological processes of malignant cells (including but not limited to proliferation and anti-apoptosis) during the maturation process in the BM was performed until recently. The studies presented in this thesis underline that the level of detail in analyzing complex cellular tissues, such as the bone marrow, is crucial to draw accurate conclusions and increase our understanding of tissue disorders. With this novel approach, it was shown that simultaneous identification of 1) the hematopoietic cell populations, 2) their respective maturation status and 3) cell biological processes of malignant cells to advance the clinical practice of MDS and AML is now finally feasible. More specifically, these studies also emphasize that one should be cautious in following the established scientific assumptions about the role of cell division and cell death in cancers. During these studies it was noticed that

the established assumption about blood- and bone marrow-borne cancers as being mainly characterized by uncontrolled cell division did not hold. The contrary seems to be true for MDS and AML. In these malignancies, cell division drastically decreased, while a high resistance to cell death is found. In AML these changes in cell division and resistance to cell death were more evident than in MDS, which indicates that these disorders are related and changes in these processes are important factors in the progression of MDS to AML. Capturing the risk of progression of MDS to AML will help to select which patients should be treated with adequate chemo- and/or immunotherapy and identify AML in an early stage to increase the chance that patients are cured of this deadly disorder. However, more studies are needed that follow the progression of these disorders over a longer time span (e.g. 5 years) and subsequently associate the parameters for cell division and resistance to cell death to the clinical behavior of these malignancies.

The recent research roadmap for myeloid malignancies from the European Hematology Association underlined that the reasons for the high resistance to chemotherapy of these malignancies are poorly understood. According to the data presented in this thesis, chemotherapy alone is unlikely to cure MDS and AML. Also, the consequences of chemotherapy can negatively affect the chances of recovery or even worsen the existing malignancy in these patients, since the malignant cells remain largely unaffected while other healthy cells that are still highly capable of cell division will be destroyed by chemotherapy. Therefore, existing shortages of functional blood cell types and subsequent complications are likely to become more severe and, more importantly, malignant blast cells (that lost their differentiation capacity) will become more dominant in the bone marrow as a result. Caution is warranted in the use of chemotherapy alone and the importance of more personalized treatment strategies, which are widely investigated currently, cannot be overestimated. These results and conclusions are of high importance to hemato-oncological researchers, hematologists and laboratory specialists and will be communicated through conference presentations about clinical cell analysis and/or hemato-oncology and publications in hemato-oncological and cytometry journals to educate these professionals.

Social impact

The uprise of treatments counteracting the resistance to cell death, such as Venetoclax, in conjunction with chemotherapy (or other novel therapies that help induce cell death such as immunotherapy) is crucial to increase the effectiveness of treatment and, ultimately, pave the way to cure patients with myeloid malignancies. Currently, indicators that predict therapy response are still under investigation. As

both cell division and resistance to cell death are important biomarkers in the response to (combinations of) these therapies, the ratio of the cell fractions for these two cell biomarkers may allow for simultaneous prediction of the response on multiple therapies. As a low Bcl-2:Ki-67 ratio indicates that cell division is favored above resistance to cell death and the malignancy may be mainly sensitive to chemotherapy, treatment with solely this therapy may be favored over the combination of chemotherapy and Venetoclax. On the contrary, the combination of chemotherapy and Venetoclax is predicted to be more suitable in patients that show a high Bcl-2:Ki-67 ratio, as the malignancy in these patients may be highly resistant to chemotherapy alone. The use of this ratio may, therefore, aid in the development of a personalized approach for treatment of these malignancies, which minimizes therapy-related complications and optimizes their effectiveness. This can provide the patients with new perspectives and an improved quality of life after receiving such a difficult diagnosis.

The complex cellular composition of the bone marrow and its disorders also pose challenges for diagnosis of these malignancies, particularly for MDS. Patients with MDS typically present themselves at the general practitioner with vague symptoms, including but not limited to fatigue, weakness, weight loss, occasional shortness of breath, frequent infections and easy bleeding and bruising. In this respect MDS patients show similarities with healthy elderly individuals, making diagnosis of MDS complex because of subjective components. The time from initial suspicion to final diagnosis of MDS is above all an emotional process for patients, that takes a maximum of several months. Patients have to undergo painful bone marrow biopsies and if the diagnosis remains unclear after the initial analysis, further biopsies are needed, resulting in a delayed conclusive diagnosis. Blood Cancer UK, a charity for blood cancer research and patient support, stated in a recent report that receiving a cancer diagnosis is heartbreaking and patients experience high levels of stress when a diagnosis is delayed for weeks, months or even years. The delay in the diagnostic process, due to the complexity of the process itself, has consequences for patients' mental and physical well-being. A faster and more sensitive diagnostic tool for MDS has the potential to contribute to less stress and a better mental health as a result of reduced waiting times for a definitive diagnosis, and adequate treatment in case of swift progression to AML, which is known for its high mortality. More accurate and straightforward diagnosis of MDS also reduces the number of unnecessary clinical visits and additional painful bone marrow biopsies. Taken together, the impact on the mental and physical health of patients with MDS can be enormous. Adequate, straightforward and objective diagnosis of MDS will be beneficial for the well-being of these patients and crucial to monitor development of AML. To advance

further implementation of these findings, a large cohort of MDS patients (and control patients) should be collected in a collaborative effort of (inter)national institutions.
As transfusion-dependence of MDS patients significantly impairs their quality of life and prognosis of survival, proper management of this severe complication is of utmost importance. Transfusion-dependence contributes to the fatigue, weakness and occasional shortness of breath that can be severely debilitating to these patients and also has severe consequences for the patient's mental and physical well-being. By combining the conventional predictor (hemoglobin levels) with the Ki-67 proliferation index, it is possible to more accurately identify whether patients will develop this severe disease complication. This allows for more adequate anticipation in clinical practice and paves the way for strategies to better stabilize this debilitating complication. This may allow the reduction of burdensome clinical visits for MDS patients that show no potential to develop transfusion-dependence in the near future, which could also lead to a reduction in healthcare costs. Furthermore, the Ki-67 proliferation index may become an additional prognostic parameter in the established international scoring systems for prognosis of MDS, which also aids in more personalized medicine for these malignancies.

Future challenges

Collectively, these findings show that biomarkers for cell division and inhibition of cell death have a high potential for a wide variety of applications during the whole clinical process of myeloid malignancies to improve the curability and quality of life for these patients. For the clinical implementation of these parameters validation in large-scale multi-center studies and over longer periods of time are necessary to confirm their diagnostic performance and predictive capabilities. Workshops for training operators and clinicians to facilitate the multicenter study are needed, while after successful validation, an advisory board with relevant stakeholders (including the patient organization and an implementation specialist) needs to discuss revision of the current diagnostic guidelines. The EuroFlow and European LeukemiaNET consortia are important groups in the field of hemato-oncology that should be represented in this initiative. In foregoing years, standardization of reagent use, flow cytometry protocols and gating procedures by for instance the EuroFlow consortium led to reducing potential discrepancies in the clinical decision making of myeloid malignancies. Organizing quality assessment rounds after validation of the Ki-67 proliferation index and the Bcl-2 anti-apoptotic can further standardize the use of these biomarkers in an international context and can ultimately allow a broad spectrum of clinical applications.