Selective targeting of the tumor cells and protection of the mesenchymal stem cells

Selektive Bekämpfung von Tumorzellen in ihrem natürlichen Milieu

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Ausgangssituation

The interaction between tumor cells and their specific microenvironment plays a central role in the generation, growth and expansion of the malignant clones and in drug resistance. While the tumor associated stromal cells are not a part of the malignant transformation process, they acquire a tumor supportive phenotype and are subjected to damage during cancer therapy. They originate from the mesenchymal stem cells (MSC) and play an important physiological role in tissue regeneration and hematopoiesis. Therefore, it is necessary to establish a model for drug testing which simultaneously demonstrate a direct effect on tumor cells while protecting the stromal component. Chronic lymphocytic leukemia CLL cells, which are dependent on the microenvironment for survival, serves in this project as a model disease for investigating these issues.

Ziele / Methoden

1. Selective targeting of the tumor cells by small molecules which target signaling events that are essential for survival of tumor cells but not the stromal cell
2. Identification of drug combinations (Immuno-chemotherapy) which effectively target the tumor cells without damaging the stromal cells or affecting their proliferation and differentiation capacity
3. Exploration of the downstream targets of drug combination and identify new potential candidates for targeted therapy
4. To Delay senescence and improve the functions of bone marrow stromal cells by hTERT transfection and generate a standardized in vitro model for drug testing.

Experimental approaches and available results:

1. Selective targeting (CLL working model):

In order to demonstrate the selective targeting of the tumor cells and protection of the stromal cells we applied an ex vivo co-culture model (Shehata et , JCI 2004, Blood 2010). This model is based on incubating CLL cells together with bone marrow stromal cells. This culture conditions prevent spontaneous apoptosis of CLL cells and allows drug testing and demonstrating the feasibility of selective targeting of tumor cells. A working model is shown in figure 1.

2. PI3-K inhibitors selectively target CLL cells
CLL cells were exposed in co-culture with bone marrow stromal cells (BMSC) to increasing concentration of pan PI3-K inhibitors (LY294002, Wortmannin and PI-103) or isoform-specific PI3-K inhibitors against PI3-K-alpha, -beta, -gamma and –delta isoforms. The effect on the viability of CLL cells and stromal cells was assessed by MTT assays and flow cytometric analysis. As shown in figure 2, the pan- and isoform-specific PI3-K inhibitors significantly decreased the viability of CLL cells in a dose dependent manner. Under the same co-culture conditions, PI3-K inhibitors did not significantly influence the viability of the stromal cells. These results suggest that the tumor cells are more dependent or addict to the PI3-K signaling cascade. Thus, confirming the feasibility of a tumor-selective targeting approach and open new option for targeted therapy of cancer.

3. Exposure of bone marrow stromal cells does not affect their phenotype:
Since exposure of stromal cells to PI3-K inhibitors did not influence their viability, extended experiments were performed to study the effect of the inhibitors on the major surface antigens which are characteristic for BMSC. As Shown in Figure 3, the PI3-K inhibitors did not influence the viability of BMSC as demonstrated by Annexin V/PI staining, and did not affect the expression of CD13, CD73 and CD105. The data further confirm the feasibility of selective targeting of the leukemic cells and that the stromal cells or tumor associated cells might be less sensitive to the inhibitors of PI3-K pathway.

4. Drug combined approach for targeting CLL cells in co-culture with bone marrow stromal cells:
CLL cells were exposed to several drug combinations and the viability of the leukemic cells and stromal cells were assessed by MTT assays. CLL cells were treated in co-culture with pan-PI3-K inhibitor (LY294002) in combination with apigenin, a casein kinase-2 (CK2) inhibitor. As shown in figure 4 (left panel) The combination of PI3-K and CK2 inhibitors was more effective and showed synergistic effect on the viability of CLL cells. Similarly, PI3-K- and CK2-inhibitors enhanced the effect of Fludarabine which is the most common current chemotherapeutic agents in CLL.

5. Prolongation of the life span of BMSC by hTERT transfection:
Since the BMSC play a normal physiological role in tissue regeneration and recovery of hematopoiesis, this part of the project aimed at enhancing the viability and the quality of those cells through overcoming the process of senescence and aging which is associated with reduced functionality. Therefore, BMSC were transfected with vector carrying the catalytic domain of

human telomerase gene (hTERT) and the effect of cell proliferation, senescence and differentiation capacity was evaluated.
As shown in figure 5, we were able to transiently induce the activation of hTERT (Figure 5 upper panel). We could also induce hTERT activity by adenoviral transfection as demonstrated by TRAP assay and this was associated with the delay in the incidence of senescence as demonstrated by ß-Gal assays (middle panel). The effect of hTERT transfection was temporary and induced proliferation of the BMSC within the first 7-14 days. This was followed by a normal process of senescence which is a major advantage to avoid immortalization of BMSC or generation of fibrotic lesions. In addition, the data demonstrate that wild type hTERT may increase the adipogenic capacity of BMSC while dominant negative hTERT significantly decreases the adipogenic differentiation capacity of these cells.

Ergebnisse

The generated data from this project to this point demonstrate the feasibility of selective targeting of the tumor cells as presented in CLL model while preserving the viability of the BMSC. The results also demonstrate efficiency of drug combination on achieving a selective targeting of the tumor cells and that the stromal cells appears to be less sensitive to the inhibition of the PI3-K and to cytotoxic compounds. Furthermore, the results point to the potential value of applying hTERT transfection to prolong survival and decrease senescence of BMSC.

Ausblick

1. Extended studies on selective targeting of the tumor cells by currently used drugs, small molecules, therapeutic antibodies as single agents and in combination to achieve an effective and selective killing of the neoplastic cells.
2. Extensive molecular investigation to identify new downstream molecules and targets for novel drug combinations to overcome the supportive effect of stromal cells and perform individualized therapy. This includes western blotting, RT-PCR, FACS, immunofluorescence studies and microarray analysis.
3. Further approaches including hTERT transfection methods using adenovirus, retrovirus and lentivirus are currently tested to prevent senescence in BMSC, enhance their functionality, hematopiesis support and differentiation capacities

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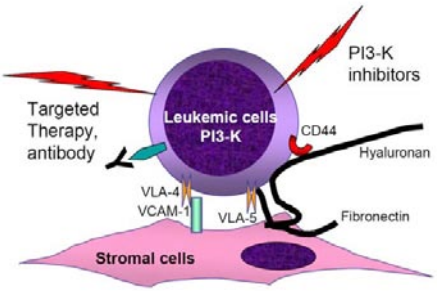


Abb. 1: Working model for selective targeting of the leukemic B cells by the combination of antibody and small molecule PI3-K inhibitors.

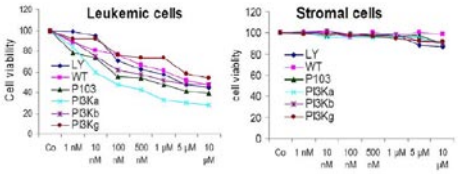


Abb. 2: Selective targeting of the leukemic cells by PI3-K inhibitors and protection of the normal stromal. A representative case of 6 individual experiments. The upper panel (MTT assays) demonstrates the selective inhibitory effect of PI3-K inhibitors on CLL cells and preservation of stromal cells. The data was confirmed by FACS analysis (middle panel) and the selective effect was visualized by Giemsa staining and light microscopy (lower panel).

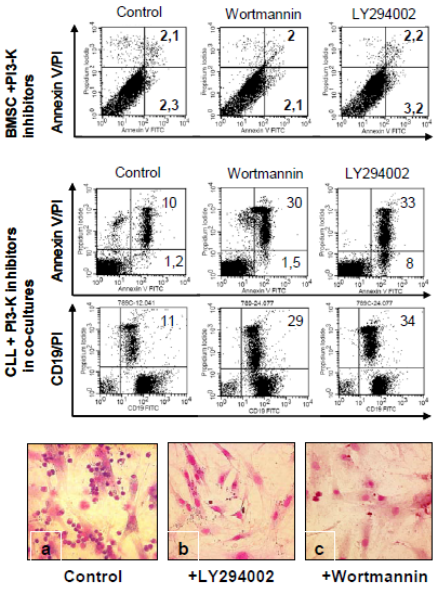


Abb. 3: Exposure of bone marrow stromal cells to PI3-K inhibitors does not affect their phenotype: Representative data of 6 individual experiments. The results demonstrate the preservation of BMSC viability as well as the expression of the major surface antigens (CD13, CD73, CD105) on the stromal cells.

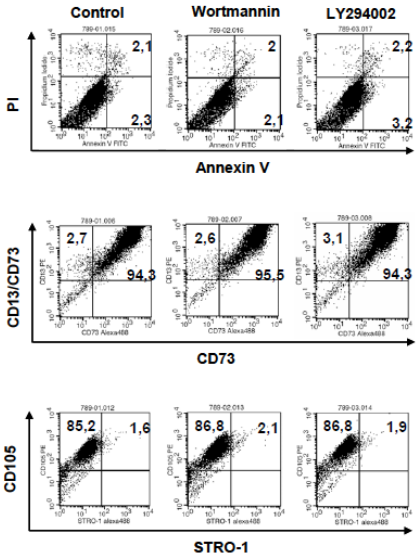
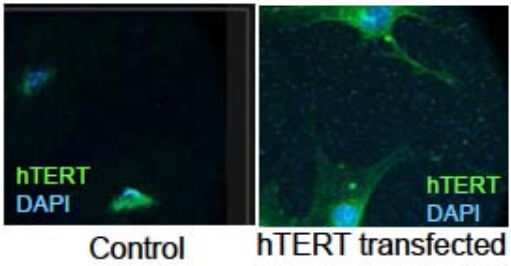
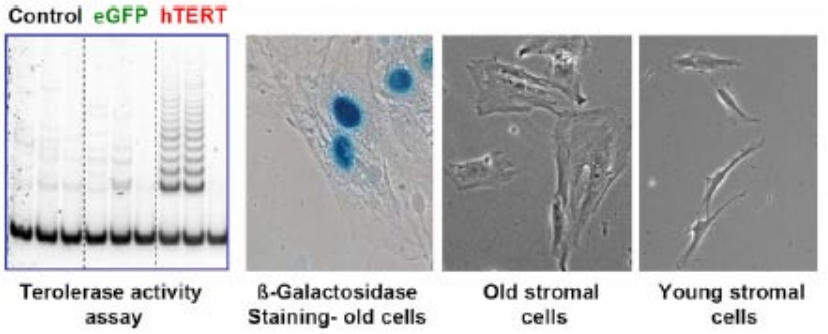


Abb. 4: Drug combination studies using PI3-K and CK2 inhibitors with Fludarbine: As shown by MTT assays, the combination of PI3-K and CK2 inhibitors had synergistic effect on the viability of CLL cells and enhanced the cytotoxic effect of Fludaranine on CLL cells. A representative case of 7 individual experiments.





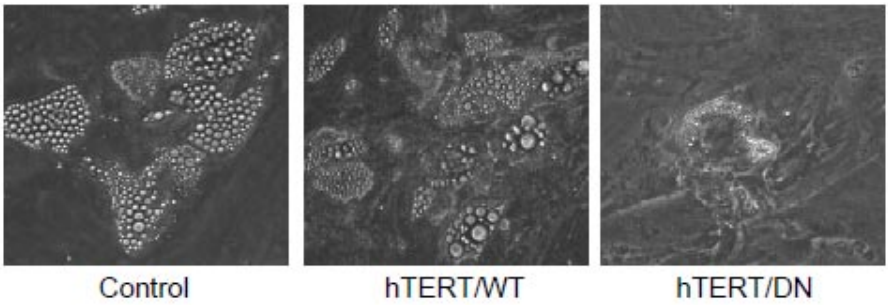


Abb. 5: Transfection of BMSC with Adenoviral vector carrying the catalytic domain of human telomerase gene (hTERT) leads to delayed senescence of BMSC and appears to be essential for the adipogenic differentiation of these cells. The figure represents data obtained from 4 long-term individual experiments.