A new treatment strategy for myeloid neoplasms

- The role of senescence in hematologic cancer

Gustav Rydström
Medicine resident, Växjö
gustav.rydstrom@kronoberg.se

Principal supervisor: Jörg Cammenga, senior Lecturer of Hematology, Lund university, Senior Attending, Department of Hematology, Scania University Hospital, Lund

Co-supervisor: Roman Galeev, Doctor of Philosophy in Medicine, Oncology resident, Scania University Hospital, Lund

Background

Despite major advances in oncology during the last decade, the mortality in patients with myeloid neoplasms (MN), such as acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), remains high. Chemotherapy is the most important treatment strategy for a majority of the patients where a selected group (younger individuals with no significant comorbidity) can be offered a bone marrow transplant – today's only available alternative to cure these conditions. However, these aggressive treatment alternatives are afflicted by multiple and severe side effects with a substantial risk of treatment related death. Today only 1 out of 4 patients with MN are alive after 5 years, mainly due to high risk of relapse and the ineligibility of the majority of older patients for curative treatments (1). Clearly, we are in need of not only more effective and tolerable treatments but also strategies to decrease the risk of relapse as well as to decrease the side effects of established treatments.

A strategy with potential to address all the challenges described above is to target a phenomenon known as cellular senescence. This is a cellular state characterized by a near-irreversible cell cycle arrest, flattened morphology, hyper metabolism and, critical in the context of cancer, resistance to apoptosis and secretion of pro-inflammatory cytokines and tissue remodelers collectively termed senescence associated secretory phenotype (SASP). Senescent cells accumulate naturally with increasing age but different stress factors speed up this process, such as chronic inflammatory conditions, toxins, UV-radiation and unhealthy life habits, e.g. alcohol overconsumption and lack of exercise. Increased number of senescent cells causes an increased level of SASP proteins, and high levels of SASP are clearly correlated with increased risk of degenerative conditions, polypharmacy, morbidity as well as mortality in humans. Additionally, the SASP itself causes senescence in a paracrine/endocrine positive feedback loop which results in an ever-increasing level of senescent cells, SASP, morbidity and frailty (2-4).

The role of senescence in cancer is however less linear. Murine studies with different cancer models have shown that the mere presence of senescent cells is associated with worse survival. Interestingly, selective elimination of senescent cells after chemotherapy increased survival compared to chemotherapy alone (5-6). A clinical study demonstrated that patients with colorectal cancer have 2,5 times more senescent cells in the surrounding stroma compared to patients with a benign adenoma

(7). At the same time an increased number of intratumoral senescent cancer cells was associated with a better prognosis in patients with stomach cancer. Interestingly, the effect of adjuvant chemotherapy did not improve survival (8). Adjuvant chemotherapy in breast cancer patients lead to an increased senescence burden and many of the acute and long-term side effects seemed to be caused by SASP factors (9).

Since a senescent cell is dysfunctional it is not surprising that senescence in cancer cells themselves results in a less aggressive cancer. Senescence in seemingly healthy cells in the tumor micro-environment is, however, a strongly negative prognostic factor where the SASP factors can facilitate tumor progression and metastasis. As demonstrated by the above-mentioned studies, the relationship between chemotherapy and senescence is complex. The common cytotoxic mechanism of action for chemotherapy is to induce apoptosis via DNA- and macromolecular damage. If the damages are not severe enough the cancer cell might become senescent. In short-term this is a beneficial result; a senescent cancer cell has lost its proliferative ability and will most likely do no harm. As a side effect, however, the chemotherapy also induces senescence among healthy cells. In the long term, this therapy related increase of senescent cells (healthy and malignant) can result in higher levels of SASP proteins and thereby, with their mitogenic properties, cause the senescent cancer cells to reenter cell division. This can result in a disease relapse which might be refractory to chemotherapy (due to resistance to apoptosis) (10). Due to these and other studies, cellular senescence is now included in the latest Hallmarks of Cancer update published in 2022 (11).

The relationship between senescence and MN is, however, less well established. Preclinical studies have shown that leukemic cells actively remodel the bone marrow niche and increase senescence in the stroma. Selective clearance of senescent niche cells increases survival in AML mouse models (12, 13). Although we lack clinical studies, given these observations it is tempting to hypothesize that elimination of senescent cells after conventional MDS/AML treatment would rid the bone marrow of the apparent pro-leukemic stimuli. Thus, targeting senescence is a very attractive strategy to address two pressing clinical needs today; high risk of relapse and severe treatment side effects of established therapy. The purpose of this study is to establish a direct relationship between the levels of senescence and clinical variables including survival in patients with AML and MDS.

Material and methods

Characterization of senescence markers in myeloid neoplasms *in vitro*

In this study we aim to measure the senescence level in patients with AML and MDS by using the concentration of SASP proteins as a surrogate marker. However, the secretome is complex and over 1000 different SASP factors have been described in literature. Which proteins that are secreted depends both on the senescence stimulus and cell type. Our first step is therefore to determine the SASP factors secreted by senescent myeloid and mesenchymal cells in response to different stimuli.

Design: Project 1 is a mechanistic *in vitro* study.

In vitro MN-model: In this project we will use human $CD34^+$ cells isolated from cord blood and human mesenchymal stem cells isolated from bone marrow aspirates.

Methods: Senescence is induced in the above-mentioned cell types using irradiation and doxorubicin as well as cytarabine, the most commonly used chemotherapeutic drugs in AML patients. The cells are treated for different periods of time and at various drug concentrations or Grays. Using flow cytometry, qPCR and ELISA the level of different senescence markers as well as known SASP-factors are then analyzed at different time points after treatment; this in order to determine the peak of senescence level. Senescence markers and SASP factors included are CD87, p16, GDF15, TNF-a, IL-1b, IL-6, CD36 and CD26. Untreated cells are used as controls. At the peak of senescence level, supernatant from the cells is isolated and subjected to external proteomic analysis (SomaScan Assay), which includes measurement of > 10 000 different proteins (REF). These results lay the foundation for our clinical study. Student's two tailed t-test (p 0,05) will be used in statistics assuming equal variance.

Ethical discussion: Since this is an *in vitro* study no ethical permission is required.

Time plan: Jan 2024 – June 2025.

Quantification of senescence in patients with myeloid neoplasms

Based on the results from project I our second step in this study is to quantify the senescence level in patients with AML and MDS and correlate it to clinical variables.

Design: Project II is a retrospective cohort study using patient blood samples stored in our regional biobanks.

Population: Patients 18 years of age or older diagnosed with myeloid neoplasms (AML or MDS) between 2012-2022 with archived plasma samples are included in this study. Otherwise, no exclusion criteria are used. The reason for not excluding individuals with comorbidities, such as rheumatic diseases and previous malignancy, is that senescence has been linked to several other conditions. This gives us an opportunity to evaluate or even quantify comorbidity in a more detailed manner. Plasma samples from healthy, age matched blood doners will serve as controls.

Method: Our research group has obtained ethical approval to access blood samples stored in our regional biobanks from the above-mentioned patients as well clinical variables from their medical records. With ELISA the level of SASP factors is measured in plasma samples collected at the time of diagnosis and, if available, after therapy. Plasma samples from healthy blood doners are used as controls. Due to a higher number of samples and high analysis costs, we only measure the most significant elevated SASP factors (based on the SomaScan Assay results from project I). Next, we obtain the following clinical variables from the patients' medical records: age at diagnosis, gender, performance status, comorbidities, presence of predictive MN related mutations, oncological therapy received, therapy related complications (side effects, the need for inpatient care) and survival. These parameters are then correlated to the senescence level. The number of available clinical senescence studies are few, why it is hard to perform adequate power analysis. However, we estimate that at least 30-35 patients per diagnosis are needed (alpha 0,05, beta 0,2), but to discover more subtle connections between senescence level and the clinical variables we hope to include at least 70 patients. At the time of writing, this study is under design and a professional statistician will be involved.

Ethical discussion: Ethical permission received. 2023-06-25 (Dnr 2023-02831-01). This is a retrospective study analyzing the senescence level in blood samples from patients with myeloid neoplasms. The patients included in this project will not be subjected to sampling or physical examination. Participants will likely not benefit from the results, since the main purpose of the project is to improve the outcome for future cancer patients. There is a risk of violating the patients' personal integrity during the process of obtaining clinical variables from medical records; therefore, all variables will be processed as pseudonymous data once obtained. Preclinical and smaller retrospective observational studies show that senescens has an important role in cancer development and it is not unlikely that treatments targeting senescence will be included in future cancer therapy. We therefore argue that the benefits from our study outweigh the potential small risk of negative effects for the study participants.

Time plan Jan 2024 – June 2026

Financing: The salary cost for this project is mainly financed by local funds from FoU Kronoberg. Material and analysis costs are covered by the research group.

References

1. Amin MB ES, Greene F, Byrd DR, Brookland RK, Washington MK, Gershenwald JE, et al. (Eds.). AJCC Cancer Staging Manual (8th edition). Springer International Publishing: American Joint Commission on Cancer. 2017.

2. Saleh T, Tyutynuk-Massey L, Cudjoe EK, Jr., Idowu MO, Landry JW, Gewirtz DA. Non-Cell Autonomous Effects of the Senescence-Associated Secretory Phenotype in Cancer Therapy. Front Oncol. 2018;8:164.

3. Schmitt CA, Wang B, Demaria M. Senescence and cancer - role and therapeutic opportunities. Nature reviews Clinical oncology. 2022;19(10):619-36.

4. Wang L, Lankhorst L, Bernards R. Exploiting senescence for the treatment of cancer. Nature reviews Cancer. 2022;22(6):340-55.

5. Demaria M, O'Leary MN, Chang J, Shao L, Liu S, Alimirah F, et al. Cellular Senescence Promotes Adverse Effects of Chemotherapy and Cancer Relapse. Cancer discovery. 2017;7(2):165-76.

6. Cortesi M, Zanoni M, Pirini F, Tumedei MM, Ravaioli S, Rapposelli IG, et al. Pancreatic Cancer and Cellular Senescence: Tumor Microenvironment under the Spotlight. International journal of molecular sciences. 2021;23(1).

7. Guo Y, Ayers JL, Carter KT, Wang T, Maden SK, Edmond D, et al. Senescence-associated tissue microenvironment promotes colon cancer formation through the secretory factor GDF15. Aging cell. 2019;18(6):e13013.

8. Zhou L, Niu Z, Wang Y, Zheng Y, Zhu Y, Wang C, et al. Senescence as a dictator of patient outcomes and therapeutic efficacies in human gastric cancer. Cell death discovery. 2022;8(1):13

9. Sanoff HK, Deal AM, Krishnamurthy J, Torrice C, Dillon P, Sorrentino J, et al. Effect of cytotoxic chemotherapy on markers of molecular age in patients with breast cancer. Journal of the National Cancer Institute. 2014;106(4):dju057.

10. Milanovic M, Fan DNY, Belenki D, Däbritz JHM, Zhao Z, Yu Y, et al. Senescence-associated reprogramming promotes cancer stemness. Nature. 2018;553(7686):96-100.

11. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer discovery. 2022;12(1):31-46.

12. Abdul-Aziz AM, Sun Y, Hellmich C, Marlein CR, Mistry J, Forde E, et al. Acute myeloid leukemia induces protumoral p16INK4a-driven senescence in the bone marrow microenvironment. Blood. 2019;133(5):446-56.

13. Abdul-Aziz AM, Shafat MS, Mehta TK, Di Palma F, Lawes MJ, Rushworth SA, et al. MIF-Induced Stromal PKCβ/IL8 Is Essential in Human Acute Myeloid Leukemia. Cancer research. 2017;77(2):303-11