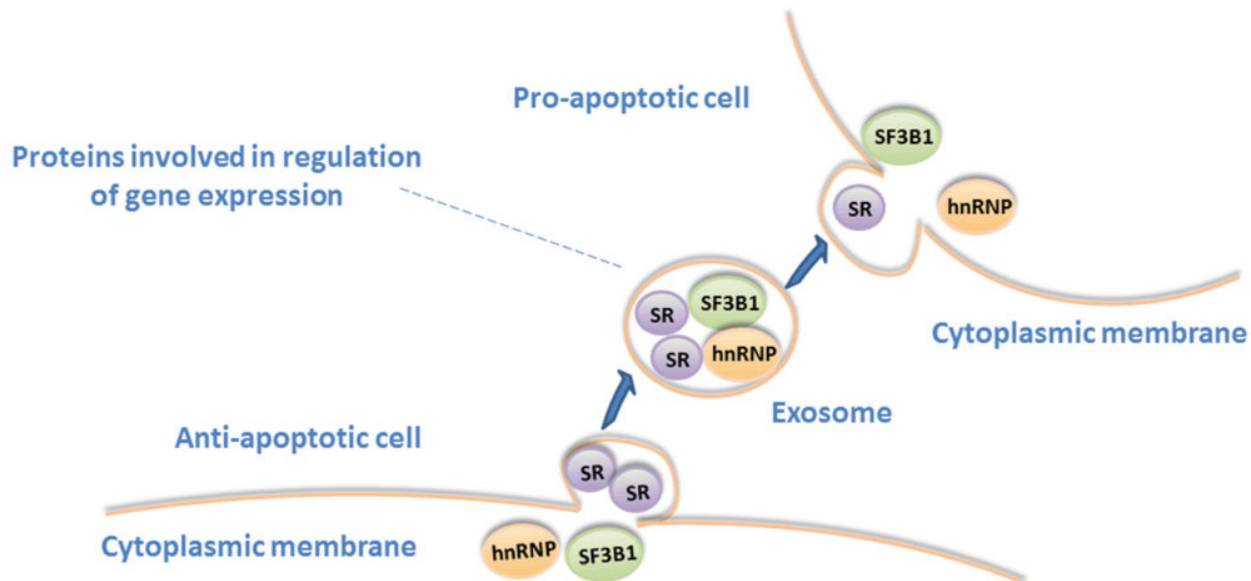


## Proteome view on how leukemia cells communicate with their environment to escape chemotherapy

Relapse continues to pose a major challenge to successful chemotherapy of adult acute myeloid leukemia (AML). Even though 80% of AML patients achieve remission, in which the bone marrow is virtually clear of leukemic cells, still almost half of them eventually relapse. One of the major causes underlying this problem is lack of proper response of leukemic cells to chemotherapy treatment.

Contemporary chemotherapeutics push cancer cells towards auto-destruction by stimulating a process called apoptosis. In a healthy body, apoptosis serves to control growth of normal cells and is thereby essential to proper organization and function of healthy tissues. In cancer the process of apoptosis is often disturbed, which can cause inability of cancer cells to auto-destruct when stimulated with chemotherapy. This ability of leukemic cells to auto-destruct can be assessed by measuring the amount of proteins which either promote or block apoptosis in each cell. Interestingly, the levels of these apoptosis regulators in leukemic cells taken from AML patients at the time of diagnosis were able to predict if the patient is going to relapse. Individuals who later on relapsed had more anti-apoptotic profile of protein expression, while those who remained in stable remission displayed pro-apoptotic profiles.



Acute myeloid leukemia cells transfer regulators of gene expression to their neighbouring cells via exosomes

In our study we investigated the changes in apoptotic profiles of AML cells during the course of chemotherapy as well as searched for novel protein factors, which might potentially influence these changes. To address these questions, we first measured the levels of apoptotic proteins not only in samples taken from AML patients at diagnosis but also after achieving remission, at later time point during remission and at relapse. In patients who did not relapse, leukemic cells had pro-apoptotic profiles at diagnosis and throughout the remission. Interestingly, in patients who did develop relapse, leukemic cells had anti-apoptotic profiles at diagnosis and at relapse but they were pro-apoptotic during remission. This was surprising since leukemic cells which remain in the patient at this point of treatment are thought to eventually repopulate the bone marrow giving rise to relapse.

In the current study apoptosis profiles were also measured in normal lymphocytes of AML patients at the same time points as for leukemic cells. Unexpectedly, we found that the level of apoptotic proteins in normal lymphocytes of AML patients were not only higher than in lymphocytes taken from healthy donors but also followed the same patterns during treatment as leukemic cells. This means that similar to AML cells normal lymphocytes of AML patients were also anti-apoptotic at diagnosis and at relapse but showed pro-apoptotic profile during remission.

The fact that both leukemic cells and normal lymphocytes shared the same ability to respond to apoptosis signals, suggests that this ability is strongly influenced by the surrounding microenvironment. In our study we showed that leukemic cells with anti-apoptotic profile when placed in the neighborhood of initially pro-apoptotic cells were able to make them anti-apoptotic.

In search of factors potentially responsible for this effect, we used mass spectrometry-based global protein profiling (proteomics) to compare proteins released by anti-apoptotic cells with those released by pro-apoptotic cells. Intriguingly, we found that proteins preferentially released by anti-apoptotic cells were engaged in various stages of gene expression instead of specifically in apoptosis regulation. Moreover, these regulatory proteins were also found in lipid vesicles released by the anti-apoptotic cells. This type of lipid structures could potentially be absorbed by bystander cells, in which their content might impact gene expression, including tendency to auto-destruct. Using confocal microscopy we showed that lipid vesicles released by anti-apoptotic cells are absorbed by pro-apoptotic cells. It remains to be determined what effect such uptake has on the functioning of the recipient cells, including the ability to auto-destruct. Further research on this matter will improve our understanding of how cancer cells communicate with their microenvironment, which is crucial for future improvements in both prognostic tools and therapy efficacy.

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## Publication

[Exosomes Secreted by Apoptosis-Resistant Acute Myeloid Leukemia \(AML\) Blasts Harbor Regulatory Network Proteins Potentially Involved in Antagonism of Apoptosis.](#)

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