

Development of drug resistance in chronic lymphocytic leukemia cells using *piggyBac* transposition

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The human body contains a large amount of different cell types. These cells are guided and regulated by a variety of intracellular and extracellular factors. In the case of deregulated cell growth and survival a tumor could appear. If these cells start to spread the disease is considered a cancer. Today, cancer is one of the most common life threatening diseases.

Leukemia is a type of cancer that affects blood or bone marrow cells. Among the different forms of leukemia, chronic lymphocytic leukemia (CLL) can be found. CLL affects B-cells that under normal conditions help our body to fight diseases by producing antibodies. CLL is a disease that primarily affects adults especially those over 60 years of age.

To aid and prolong the life of CLL cancer patients, different types of chemotherapies have been developed. Among these, the drugs chlorambucil and fludarabine are commonly used. However, nowadays, chemotherapies are often combined with monoclonal antibodies to increase the treatment efficiencies for the disease.

Unfortunately, these treatments cannot in all cases provide a cure to the disease. A common problem is that the disease develops resistance against previously used treatments. The most common way that resistance develops is because of mutations occurring in deoxyribonucleic acid (DNA). The mechanisms used by cancer cells to escape the effects of the cytotoxic drug are of great interest for understanding the disease in more detail and to subsequently be able to develop new treatments against it.

In order to find out the mechanisms active in the development of drug resistance, we wanted to recreate a situation under which resistance could occur. To be able to introduce genetic changes into the genome we used a transposon system. Transposons are DNA elements that can be moved around in the genome by an enzyme and thereby mutate the DNA within the cell. This system is called the *PiggyBac* system.

By using a cell-line that was developed from a CLL patient in combination with transposon mutagenesis we could detect cells that have growth advantages under drug selection conditions. When measuring the sensitivity of mutated cells selected in fludarabine compared to wild type cells, a significant increase in the half maximal inhibitory concentration (IC_{50}) could be detected. This strong difference could however not be detected in chlorambucil selected and mutated cells. The increased resistance in transposon mutated and fludarabine selected cells shows that the conditions responsible for resistance have been successfully recreated. By analyzing the insertion point of the transposons we may in the future be able to find the genes responsible for the resistance.