Stem cell defects in patients with lymphoma

PhD thesis

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Danish summary (dansk resumé)

Lymfom er en kræftform i det hæmatopoietiske system som rammer patienter i alle aldre, men incidensraten stiger kraftigt med øget alder. I Danmark diagnosticeres omkring 1500 patienter per år med lymfom og de fleste patienter har god effekt af førstelinjebehandling. Dog vil en væsentlig del af patienterne få tilbagefald, svære bivirkninger til behandling og/eller dø på grund af lymfom eller terapi-relaterede årsager.

Hæmatopoietiske stamceller giver kontinuerligt ophav til blod og lymfoidt væv. Disse stamceller kan erhverve DNA mutationer som fører til en skæv udvikling af abnorme blodceller, også kaldet klonal hæmatopoiese. Klonal hæmatopoiese er et almindeligt aldersrelateret fænomen som findes i både raske og syge individer. Forekomsten og de kliniske konsekvenser af stamcelle-defekter i patienter med lymfom har kun været sporadisk undersøgt indtil nu. Derudover har det været mistænkt at lymfomtilbagefald kunne stamme fra en 'lymfomstamcelle'. Disse cellers karakteristika er dog uklart beskrevet.

I artikel 1 har vi analyseret niveauerne af mikroRNA molekyler i tumorer fra patienter med mantle cell lymfom, der har været behandlet i de nordiske MCL2 og MCL3 studier. Vi fandt at ekspressionen af miR-18b var stærkt forhøjet i patienter, som døde af lymfekræft. Derudover fandt vi, at induceret ekspression af miR-18b i cancercellelinjer hæmmede cellevæksten, hvilket indikerede behandlingsresistens og en stamcelle-lignende fænotype. I artikel 2 har vi gennemgået den nuværende viden om lymfomers oprindelse og diskuteret den mulige eksistens af 'lymfomstamceller'. I artikel 3 har vi undersøgt, om der var erhvervede (somatiske) mutationer i sorterede $CD34^+$ stamceller fra patienter med lymfom. Vi identificerede stamcellemutationer, som primært var kendt fra myeloid cancer, i 30,5% af patienterne. I studie 4 undersøgte vi klonal hæmatopoiese i en national populations-baseret kohorte af patienter med lymfekræft, som fik autolog stamcelle transplantation. Vi fandt at mutationer i gener involveret i signalvejen for DNA-reparation (*PPM1D*, *TP53*, *RAD21*, *BRCC3*) var forbundet med dårlig overlevelse i en multivariat analyse. Desuden fandt vi at klonal hæmatopoiese i almindelighed var associeret med terapi-relateret leukæmi, men ikke uafhængigt associeret med død eller andre komplikationer. I artikel 5 undersøgte vi udviklingen af klonal hæmatopoiese på forskellige tidspunkter af førstelinjebehandlingen for mantle celle lymfom. De muterede kloner voksede kraftigst under behandling med kemoterapi, mens væksten aftog efter afsluttet behandling.

Studierne i denne afhandling bidrager med viden om 'lymfomstamcelle' teorien og om samspillet mellem behandling og hæmatopoietiske stamcelledefekter hos patienter med lymfom.

English summary

Lymphoma is a cancer of the hematopoietic system that affects patients of all ages, but the incidence rate increases significantly with age. In Denmark approximately 1500 patients per year are diagnosed with lymphoma, and most patients respond to first-line treatment. However, a substantial fraction of patients experience relapse, severe treatment side effects and/or death due to either lymphoma or therapy-related causes.

Hematopoietic stem cells continuously give rise to all blood and lymphoid tissue in the human body. These stem cells can acquire DNA mutations which lead to a skewed formation of aberrant blood cells, called clonal hematopoiesis. Clonal hematopoiesis is a common age-related phenomenon found in both healthy and diseased individuals. The occurrence and clinical consequences of these stem cell defects in patients with lymphoma has so far only been sparsely investigated. Additionally, it has been suspected that lymphoma relapses originate from a 'lymphoma stem cell'. However, the characteristics of these cells are only vaguely described.

In article 1, we analyzed the expression of microRNA molecules in tumors of patients with mantle cell lymphoma treated in the Nordic MCL2 and MCL3 trials. We found that the levels of miR-18b was highly increased in patients who died of lymphoma. Furthermore, we found that induced expression of miR-18b in cancer cell lines lead to a dormant growth pattern, indicative of therapy resistance and a stem cell-like phenotype. In article 2, we reviewed the current knowledge on the origin of lymphomas and potential 'lymphoma stem cells'. In article 3, we analyzed sorted $CD34^+$ stem cells from patients with lymphoma for acquired (somatic) mutations. We identified stem cell mutations, which were predominately known from myeloid cancers, in 30.5% of patients. In article 4, we investigated clonal hematopoiesis in a national population-based cohort of lymphoma patients undergoing autologous stem cell transplant. We found that mutations in genes of the DNA repair pathway (*PPM1D, TP53, RAD21, BRCC3*) were independently associated with inferior overall survival in multivariate analysis. Additionally, we observed that clonal hematopoiesis in general was associated with therapy-related leukemias, however, it was *not* independently associated with other poor outcomes. In article 5, we examined the development of clonal hematopoiesis mutations at several time points during first-line treatment for mantle cell lymphoma. Mutated clones expanded rapidly during chemotherapy, but their growth declined after the end of therapy.

In conclusion, the studies in this thesis contribute with knowledge regarding the 'lymphoma stem cell' theory and the clinical interplay of treatment and hematopoietic stem cell defects in patients with lymphoma.