Comment on Allinne et al, page 2044

**Cyclin D1 transcriptional activation in MCL**

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In this issue of Blood, Allinne et al propose the nucleolin-dependent activation of the translocated \textit{CCND1} allele in mantle cell lymphoma (MCL) because of its relocation to a transcriptionally favorable area in the perinucleolar region.\(^1\)

MCL is a CD5-positive mature B-cell neoplasm characterized by the t(11;14)(q13;q32) translocation leading to overexpression of cyclin D1, which is not expressed in normal B lymphocytes.\(^2\) This hallmark translocation is considered to be the primary genetic alteration of MCL and is found in virtually all cases. Although it may be an essential hit for the initiation of tumor development, it does not seem sufficient. This is mainly because of the weak oncogenicity of cyclin D1, which requires the cooperation of other genetic events to fully transform the lymphoid cells.\(^3\) Several molecular studies have identified additional alterations in components of cell cycle regulation, DNA damage response, cell survival pathways, NOTCH and nuclear factor-κB pathways, and chromatin modification machinery.\(^2,5,6\) These secondary molecular alterations are frequently associated with chromosomal gains and losses that target important genes as well as with an accumulation of somatic mutations. During the past 2 decades, much effort has been spent on deciphering the spectrum of these secondary genetic alterations in MCL.\(^7\) However, little is known about the molecular causes of the transcriptional activation of \textit{CCND1} in MCL cells.

In MCL, the t(11;14) seems to occur in pre-B lymphocytes of the bone marrow. The consequence of this rearrangement is that 1 \textit{CCND1} allele (located at 11q13) becomes juxtaposed to the potent intronic enhancer (E\textsubscript{μ}) of IgH (located at 14q32). Although the distance between the regulatory E\textsubscript{μ} element and the \textit{CCND1} gene is several hundred kilobases, it is believed that the mechanism responsible for the transcriptional activation of \textit{CCND1} is due to the action of this potent IgH enhancer.

The first studies on the transcriptional activation of the \textit{CCND1}-translocated allele observed DNA hypomethylation, histone hyperacetylation, and especially binding of RNA pol II (PolIII) to the \textit{CCND1} promoter region and to the regulatory IgH region, as detected by chromatin immuno precipitation (ChIP).\(^6\) Noteworthy is that the same group provided additional clues for the \textit{CCND1} activation in MCL cells, suggesting a functional role for \textit{CCCTC} binding factor (CTCF) and nucleophosmin (NPM).\(^7\) In cells carrying the t(11;14), CTCF and NPM were both associated with the IgH regulatory region and the \textit{CCND1} promoter, suggesting that CTCF could play a role in tethering both E\textsubscript{μ} and the \textit{CCND1} promoter in the nucleolar periphery through its interaction with NPM.

With these previous observations in mind and with the knowledge that in the nucleus the chromosomes occupy specific regions or territories as well as that gross genetic alterations and translocations may cause changes in the nuclear localization, Allinne et al\(^1\) have gone a step further. The authors have investigated the changes in the nuclear localization of both the intact and rearranged \textit{CCND1} alleles in MCL with the aim of determining the molecular mechanism triggering \textit{CCND1} activation in the translocated allele. They carefully measured the position and distance of the translocated and nontranslocated \textit{CCND1} alleles by 3-dimensional fluorescence in situ hybridization and observed that the translocated allele is positioned within a perinucleolar area, whereas both the normal IgH and \textit{CCND1} loci lay...
further away from the nucleolus than their translocated counterparts (see figure). The relocalization of the translocated \textit{CCND1} allele occurs in a nuclear region with high concentrations of active PolII molecules and a potent transcriptional activator, LR1, formed by heterodimers of nucleolin and HnRNP-D. Nucleolin is very abundant in the nucleolus and, among other functions, is implicated in transcriptional regulation. In contrast, the nontranslocated \textit{CCND1} allele did not colocalize with PolII transcription clusters.

Interestingly, of the 2 putative CTCF-binding sites near \textit{CCND1}, the closest to the gene is highly occupied by CTCF in MCL cell lines compared with normal lymphocytes. This finding suggests that upstream enhancer elements might not play a role in \textit{CCDN1} activation because they may be blocked by the CTCF-bound insulator. Furthermore, the authors propose that CTCF itself may play a role in \textit{CCDN1} activation, although they did not provide further evidence supporting this interesting hypothesis. Elegantly, the authors show that there are 3 LR1-binding sites in \textit{CCND1} that have enhancer activity. Thus, the high concentration of LR1 and its binding to the LR1 sites of \textit{CCND1} might be the reason \textit{CCDN1} is activated in these cells. Additionally, as a proof of concept, the authors performed ChIP-on-chip experiments with an antinucleolin antibody and confirmed the interactions of CyclinD1 protein and nucleolin. Furthermore, this experiment also revealed a high concentration of lysine 9–acetylated histone H3 at the \textit{CCND1} promoter, an expected result confirming that the translocated \textit{CCND1} allele in these cells is transcriptionally active (see figure). In addition, the treatment of the MCL cell lines with a histone deacetylase inhibitor perturbed the overall nuclear structure and dramatically reduced the transcription levels of \textit{CCND1}.

The authors also extended the relocalization studies of the translocated allele to MYC translocation in an endemic Burkitt lymphoma cell line with the (8;14) translocation. By 3-dimensional fluorescence in situ hybridization, they found a similar relocalization of rearranged alleles to the perinucleolar territories, suggesting that similar transcriptional mechanisms may lead to the activation of \textit{MYC} in this translocation. In fact, nucleolin seemed to be able to activate the transcription of \textit{MYC} more efficiently than in the case of \textit{CCND1}.

Overall, these observations shed light on the mechanisms leading to transcriptional deregulation in common chromosomal oncogenic translocations in B-cell lymphomas being the translocated allele activated by its relocalization to nucleolin-rich regions of the nucleus.

**Conflict-of-interest disclosure:** The author declares no competing financial interests.

**REFERENCES**


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**LYMPHOID NEOPLASIA**

Comment on Yakimchuk et al, page 2054

How do estrogens control lymphoma?

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In this issue of Blood, Yakimchuk and colleagues show that estrogen receptor β (ERβ) signaling can act tumor-suppressive predominantly through the regulation of genes by ERβ in the tumor, not in the microenvironment, and point out new therapeutic strategies.1

An intriguing discovery in hematologic oncology has been that women display a lower incidence of B- and T-cell lymphomas than men and female patients have a better prognosis than male patients.2,3 Although female reproductive hormones were suspected early to play a role in this phenomenon, the precise underlying mechanisms remained enigmatic. Lymphomas are still not generally perceived as hormone-controlled; however, several strong hints now point at a protective function of estrogens in lymphomagenesis. Clues came initially from epidemiological studies.2 For example, a population-based

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**ERβ** in Lymphoma cell

Effects of ERβ agonists on lymphomas.
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