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The origins of ALK translocations

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1. ABSTRACT

Translocations involving the *anaplastic lymphoma kinase* (*ALK)* gene locus on chromosome 2p23 were first described in anaplastic large cell lymphoma (ALCL). Although most commonly fused to the *nucleophosmin* (*NPM1*) gene on chromosome 5q35, which results in the t(2;5)(p23;q35)/*NPM1-ALK* translocation, several other *ALK* translocation partners have meanwhile been identified. Furthermore, apart from ALCL, *ALK*-involving translocations have been described in other hematopoietic and non-hematopoietic cancers. However, despite a rapid increase in literature on the nature and tissue distribution of *ALK*-translocations, much less is known about the mechanisms of formation of these translocations. The emergence of translocations has been linked to the transcriptional activity of the respective genome regions, reorganization of the chromatin and activation of the DNA repair machinery. In this review we discuss mechanisms and implications of formation of ALK-translocations.

2. INTRODUCTION

Chromosomal translocations are a defining feature of cancer cells and their role for initiation and maintenance of the transformation process has been particularly well documented in hematopoietic malignancies. One prominent recurrent translocation, identified in human T cell-derived ALCL more than 20 years ago, is t(2;5)(p23;q35), leading to constitutive activation of ALK. Meanwhile, many other ALK-involving translocations have been identified, and ALK-inhibition is a promising treatment strategy for various ALK-translocation$^+$ malignancies. Despite obvious clinical applications and potential treatment strategies based on these findings, only little is known regarding the molecular and cellular mechanisms leading to ALK-translocation formation. In the first part of this review we discuss current concepts of translocation formation followed by a second part summarizing the knowledge on the origin of *ALK*-involving translocations.

3. MECHANISMS OF TRANSLOCATION FORMATION: IMPLICATIONS FOR THE OCCURENCE OF *ALK*-INVOLVING TRANSLOCATIONS

3.1. General insights into the mechanisms of translocation formation

The genesis of a chromosome translocation requires the formation of double-strand breaks (DSBs) in at least two chromosome sites. Failure of the cellular DNA damage response (DDR) in repairing these lesions may allow their physical juxtaposition and the illegitimate misjoining to create aberrant fusions, i.e. chromosomal translocations. As a consequence, it is fair to predict that processes that influence the occurrence of DSBs or affect

their motion within the three-dimensional nucleus, their physical contact or their illegitimate joining, may influence the frequency of translocations. Recent studies have delineated several mechanistic steps in translocation biogenesis, and it has now become clear that the non-random positioning of chromosomes, the DDR pathways, the transcriptional activity and the epigenetic landscape are important factors in determining the selection of the translocating partners and the frequency of translocations (1).

Accumulating evidence over the last decades suggests that transcription is a driver of translocation formation in several ways. First, studies have implicated active transcription to directly influence the occurrence of breaks in the genome. For example, it has been shown that transcription generates mechanical forces leading to DNA supercoiling and torsional stress which can be relieved by the action of topoisomerases (2, 3) and, importantly, these topoisomerase-induced breaks appear to be necessary for maximal transcriptional output of specific genes (4). However, they of course generate opportunities for the formation of persistent breaks, which can be precursors of translocations. In addition, the formation of three-stranded nucleic acid structures comprised of a nascent RNA hybridized with the DNA template, which form normally during transcription (R loops), can induce chromosome breakage and genomic instability when interfering with DNA replication (5, 6). These studies suggest that the local formation of breaks within transcribed genes is an integral part of transcription and may account for increased occurrence of translocations between transcribed genome sites (Figure 1A). In support of this idea, recent genome-wide translocation-capture studies in B-lymphocytes have identified translocation break points to be frequently positioned near transcription start sites of active genes (7, 8). In line, early replicating fragile sites (9), mapped in more than 50% of the recurrent genome rearrangements in human diffuse large B cell lymphoma, are enriched in highly transcribed regions, and their fragility has been found to correlate with transcriptional activity (9).

A different transcription-related mechanism that contributes to the formation of chromosome translocations involves the formation of site-specific breaks through the recruitment of the activation-induced cytidine deaminase (AID). AID is the cytidine deaminase that initiates class switch recombination (CSR) and immunoglobulin somatic hypermutation (SHM) by deaminating cytidine residues in ssDNA, which then can be processed by different DNA repair pathways to produce mutations or DSBs (10). It has recently been shown that AID interaction with Spt5, a factor associated with stalled RNA polymerase II (Pol II) and single stranded DNA (ssDNA), facilitates AID recruitment to its targets (11). In addition to diversifying the antibody repertoire, AID-induced breaks contribute to malignant transformation by initiating chromosome translocations in lymphocytes (12). In an analogous fashion, AID co-recruitment with liganded androgen receptor (AR) to AR-binding DNA sequences sensitizes them to DSB breaks, leading to the formation of prostate-cancer-specific translocations (13). In line, AID deregulation has been associated with *H. pylori* infection and gastric cancer occurrence (14). Collectively, these studies suggest that binding of the transcription machinery may predispose genome regions to breakage that may lead to the formation of cancerous translocations.

Further evidence supporting a distinct mechanistic role of transcription in the formation of translocations comes from studies showing that transcription can influence the spatial proximity of translocation partners. Protein-coding gene transcription occurs at discrete, immobile, specialized sites called transcription factories (15, 16). These factories are typically evenly distributed across the nucleus and seem to act as centers of transcriptional activity, in which several genes can be transcribed concomitantly (17-19). Although it has been suggested that this congregation may serve to increase the concentration of transcription factors and thus enhance transcription efficiency, recent studies have raised the possibility that clustering of active genes to shared transcription factories may be detrimental in that it contributes to the formation of chromosome translocations by retaining potential translocating partners in close spatial proximity (Figure 1B). Several recent studies support this notion. It has been shown that the proto-oncogene Myc and the highly transcribed IgH gene, which are frequently translocated in various malignant lymphomas, are rapidly relocated into shared common transcription factories during gene induction in B lymphocytes (18). Moreover, in a cellular model that recapitulates translocations between the androgen-dependent TMPRSS2 gene and the transcription factors ERG or ETV1, which are found translocated in approximately 50% of human prostate cancer (20), transcriptional activation by the androgen-receptor-dependent signalling promotes the chromosome interactions of these partners (13). These studies suggest that active transcription is able to influence the probability of translocation formation by promoting the physical juxtaposition of potential translocation partners.

3.2. The origin of ALK-translocations

3.2.1. What determines the choice of ALK translocation partners?

Since the initial discovery of t(2;5)(p23;q35) and the description of its translocation product *NPM1-ALK* (21, 22) an increasing number of *ALK*-translocations has been described. Given that the nature and distribution of *ALK*-translocations are extensively discussed in other articles in this issue, we will not review them here. However, we want to point out that, although *ALK*-translocations are found in various tumor entities, the *ALK* partner

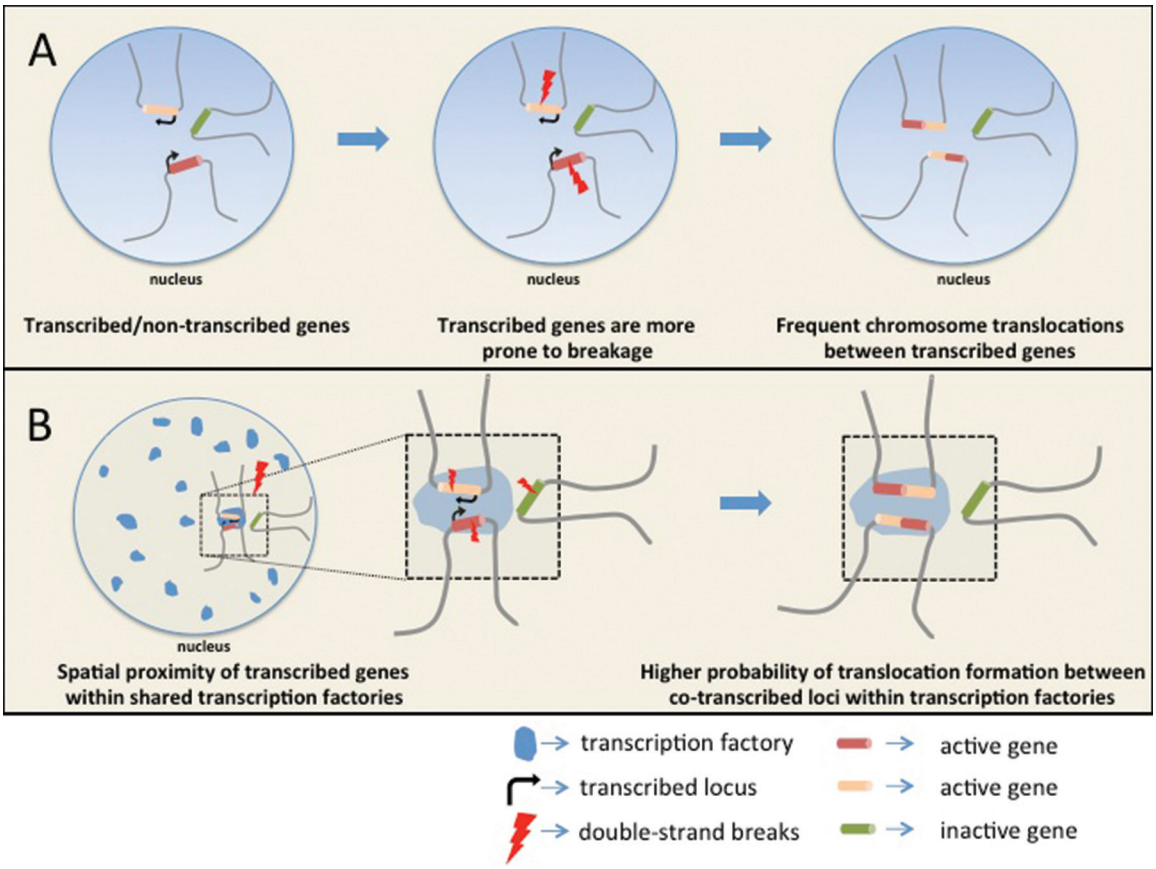

Figure 1. Transcription as a driver of translocation formation. (A) The binding of the transcription machinery to active genes (orange and red) predisposes these genome sites to breakage (red lightning bolt) and thus, makes them more prone to translocations. (B) Active genes (orange, red), co-transcribing in a shared transcription factory (blue), are retained in spatial proximity and therefore, when concomitant DSBs occur (red lightning bolts), there is a higher probability to be involved in chromosome translocations compared to the distal, transcriptionally inactive gene (green).

selection for at least a number of these translocations is highly cell type-specific (23, 24). For example, *NPM1-ALK* is lymphoma-specific and not found in solid tumors, whereas *EML4-ALK* is present in lung cancer but usually not in lymphomas. Regarding the tumor type-specificity of these translocations the obvious question arises which processes predispose for selection of the respective translocation partners in ALK-translocation^{+} malignancies. Mechanisms of translocation formation have intensively been studied for translocations occurring in B cell lymphomas such as Burkitt´s lymphoma, e.g. *IgH* locus involving translocations like *IG-MYC* (for a recent review see (25)). In these cells, a machinery involving AID and RAG proteins is actively initiating DSBs in the *IG* gene loci, and their activity is intimately linked to the occurrence of *IG*-translocations. Despite the tight control of genes involved in these processes, their involvement in the formation of DSBs has also been documented in the translocation formation in solid tumors such as prostate cancer (13) and in targeting of non-*IG* genes involved in recurrent translocations in lymphoid cells (26). Focusing on ALCL, AID or RAG proteins seem not to be expressed at detectable levels and detailed data on epigenetic alterations or structural elements predisposing for DSBs, such as fragile sites or alterations of the chromatin structure at the putative breakpoint regions, are not available. The presence of such alterations and their impact on ALK-translocation formation should be investigated in future studies. Furthermore, apart from a common intronic *ALK* breakpoint region (23), breaks in an *ALK* exon have also been reported in ALCL (27). We have to admit that the exact mechanisms accounting for breaks on 2p23 and the different choice of ALK-translocation partners in distinct cell types are largely unknown. One common feature of ALK translocations is that in most of the physiological counterparts of the respective ALK-transformed cells, ALK is not expressed (24). In these cells, a selection process for translocation partners with ongoing promoter activity has to be assumed. How frequent other non-productive ALK-translocations, which do not result in an ALK activation, occur during the transformation process might be answered in the future by high-throughput analyses such as the recently developed high-throughput, genome-wide translocation-capture sequencing methodologies (7).

3.2.2. Transcriptional alterations and chromosome positioning in ALCL and their link to *NPM1-ALK* translocation formation

Among the above outlined mechanisms promoting translocation formation, transcriptional alterations and spatial reorganization of the translocation-involved chromosomes might directly favour the occurrence of ALK-translocations. Since among ALK-translocation$^+$ malignancies, ALCL might represent the most appropriate model system, we focus in the following paragraphs on ALCL. In ALCL, the t(2;5)(p23;q35) is found in approximately 50 – 60 % of cases. ALK$^+$ and ALK$^-$ ALCL share morphological, immunophenotypic and also molecular characteristic features (28) and, at the level of the transcriptome, gene expression differences between ALK$^+$ and ALK$^-$ ALCL are largely determined by an ALK signature in ALK$^+$ ALCL (29). Despite known differences with respect to the genomic landscape (30, 31) it could be assumed that ALK$^+$ and ALK$^-$ ALCL share (apart from ALK-induced gene expression changes) a common, fundamental cell-characteristic gene expression program. Importantly, as outlined above, the transcriptional landscape is a major determinant of frequency and occurrence of chromosomal translocations (1, 8, 32). Of particular interest is the notion that transcriptional activation of genes alters positioning of chromosomes in the nuclear space, brings putative translocation partners into close spatial proximity in a cell type-specific manner, and predisposes them for translocation formation (13, 33, 34). This concept has been described by the “contact-first” model for chromosomal translocations (35). Again, pivotal evidence for the link between translocation frequency and nuclear distance of the respective partner chromosomes comes from the investigation of *IG*-translocations in B lymphoid cells (18, 34). Importantly, DSBs guided by such cell type-specific transcriptional alterations (either induced by a physiological, cell-stage specific transcriptional program or maintained by e.g. aberrantly activated transcription factors during an early transformation process) are likely explanations for the reported cell type-specificity of various ALK-translocations.

In our previous work we have assumed that, due to the close relationship of ALK$^+$ and ALK$^-$ ALCL, this lymphoma type might be a valuable model to study mechanisms of ALK-translocation formation (36). Focusing on the most commonly found t(2;5) (p23;q35)/ *NPM1-ALK* translocation we have identified a series of relatively proximal genes to the putative breakpoints on chromosomes 2p and 5q showing altered expression level and, in part, copy number alterations of the respective gene loci in ALCL. These genes include the oncogenic tyrosine kinase receptor *CSF1R*, the oncogenic AP-1 transcription factor member *FRA2* and the helix-loop-helix protein *ID2*. All these genes have important functions in ALCL with or without the characteristic t(2;5) (p23;q35)-translocation, suggesting that they support the transformation process of both ALCL subtypes, even in the absence of an ALK translocation (36). Although not performed with an extensive number of cell lines, it could be shown that only in cells with an aberrant transcriptional activation of these genes, including ALK$^-$ ALCL, the translocating chromosomes 2p and 5q were found in close spatial proximity in the nuclear space. Importantly, in cells with respective transcriptional alterations and spatial proximity of 2p and 5q, the t(2;5)(p23;q35)/*NPM1-ALK* translocation was induced following application of genotoxic stress (36). These data suggest that chromatin changes, most likely induced by ALCL-specific transcriptional alterations, trigger changes in positioning of the translocation-involved gene loci and predispose them for the formation of *NPM1-ALK* translocations. Thus, such a cell-type- or developmental-stage-specific transcriptional program with respective changes of the chromatin structure predisposing for at least the majority of occuring chromosomal translocations (37) might be the major source for the choice of *ALK* translocation partners in distinct ALK-translocation$^+$ malignancies. This hypothesis has to be addressed for other ALK-involving malignancies and translocation types in future studies. Furthermore, a shared transcriptional program of ALK$^+$ and ALK$^-$ ALCL cells, as e.g. demonstrated for overlapping activities of STAT3 or AP-1 transcription factor programs, and subsequently overlapping transcription factories, might be a likely explanation for the spatial proximity observed for breakpoint proximal genome regions also in ALK- ALCL (36) (see also above).

3.2.3. Experimental approaches to the mechanisms and consequences of ALK-translocations

Structural and functional alterations occurring before the formation of a respective translocation and those induced by the translocation event itself are highly complex. This raises the question of how accurately the experimental settings based on ectopic or transgenic expression of the ALK-involving translocations mirror the biological consequences of translocation formation. Furthermore, key requirements predisposing the genome for the generation of specific ALK-translocations in distinct malignancies (e.g. NPM-ALK in ALCL or EML4-ALK in NSCLC (23, 24)) might not be reflected accurately in a chosen cell type for such experiments. In particular, direct and dominant effects of the respective fusion protein-mediated activation patterns of e.g. signaling pathways will be properly covered (23, 24). However, other important features might be missed including the resulting heterozygosity of the translocation partners, which might itself support malignant transformation (38). For NPM-ALK-induced lymphomagenesis, the effects of *NPM1* heterozygosity have been started to be investigated (39). Furthermore, transcriptional and epigenetic changes of breakpoint-surrounding genes, cell type-specific ‘background transcription factor activities’ cooperating with ALK-transgene activities

and transgene expression levels are difficult to be incorporated in most settings. Some of these issues might be resolved in the future by more sophisticated experimental approaches such as the use of designer endonucleases like transcription activator-like effector nucleases (TALENs) or RNA-guided nucleases like the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system, by which specific genomic alterations can be introduced experimentally (40, 41). Interestingly, endogenous *NPM1* and *ALK* gene loci have already been targeted by TALENs in non-ALCL lymphoid and epithelial cells with subsequent generation of *NPM1-ALK* translocations resembling those observed in ALCL (42). Apart from the resulting heterozygosity of the affected gene loci, the regulation of the resulting fusion-gene from the endogenous *NPM1*-promoter represents a further advantage of this approach. Finally, the cell of origin of ALK-bearing malignancies like ALCL is, despite recent progress, still a matter of debate (28, 43) and therefore the cellular background and developmental stage in which ALK-translocations occur remain to be elucidated.

3.2.4. ALK-translocations in primary cutaneous ALCL

In the group of $CD30^+$ cutaneous lymphoproliferative disorders (CD30-CLD) primary cutaneous ALCL (cALCL) may arise from CD30-CLD such as lymphomatoid papulosis (LyP) in up to 20% of cases. Furthermore, cALCL and LyP often share pathologic features (44, 45). We have previously demonstrated that at least some of the breakpoint-surrounding genes, like FRA2 or ID2, which show a specific, biologically important deregulation in ALK^+ and ALK^- ALCL (see also above), are up-regulated in LyP as well (36). Given the link between deregulation of these breakpoint-surrounding genes and spatial proximity of the putative translocation partners on chromosomes 2p and 5q as well as the induction of *NPM1-ALK* translocations in *in vitro* studies (36), it might predicted that at least occasionally ALK translocations might occur in ALCL limited to the skin. Although initially reported as restricted to systemic ALCL, ALK^+ primary cutaneous ALCL (cALCL) have been reported (46-48). Only recently, ALK-translocation$^+$ cALCL have unambiguously been demonstrated and found at a remarkable frequence (49). Due to the fact that the analysis of ALK expression has not routinely been performed in most studies on cALCL, the current percentage of ALK-translocation$^+$ cALCL might be an underestimation. These data highlight that the close relationship between ALK^+ and ALK^- ALCL also holds for ALCL restricted to the skin. Based on these findings several questions arise. The most relevant being what the frequency and causative events of these ALCL subtype translocations are, the reason why these ALK^+ cALCL do not progress to systemic ALCL and by which factors their excellent prognosis (49) is determined.

4. SUMMARY AND PERSPECTIVE

In contrast to the increasing amount of data regarding the nature and tissue distribution of ALK-translocations, only little is known about the mechanisms of formation of these translocations in the respective malignancies. With ALK inhibitory compounds we have powerful tools in our hands to treat ALK-induced malignancies (50, 51). Therefore, why should we further study the mechanisms of ALK-translocation formation? For several reasons the elucidation of translocation formation in ALK-translocation$^+$ malignancies is not only of interest for understanding the pathology of the disease, but is also relevant for the development of treatment approaches, independent of or complementary to ALK-inhibition. First, as observed for other small compound inhibitors, ALK-translocation bearing malignancies develop resistance towards ALK inhibitors (52, 53), which makes the search for alternative targeted treatment strategies mandatory. Second, understanding the mechanisms of ALK translocation-formation might lead to identification of druggable target structures in ALK-negative, closely related malignancies as demonstrated for ALK^+ and ALK^- ALCL (36). Third, the presence of sibling ALCL types, with and without ALK translocations, suggests the use of ALCL as model system to supplement other experimental systems like yeast and to investigate the mechanisms of translocation formation.

The study of ALK translocations finds itself at a powerful confluence of recent developments. Advances in the clinical characterization of ALK-containing cancers is progressing rapidly with novel therapeutic approaches being developed. At the same time, progress is being made in the elucidation of the basic mechanisms of how chromosome translocations form in intact cells and tissues. ALK-translocations, and the cancers that contain them, are a promising experimental system to understand translocation formation and their consequences across the basic to clinical spectrum.

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Abbreviations: AID: activation-induced cytidine deaminase; ALCL: anaplastic large cell lymphoma; ALK: anaplastic lymphoma kinase; AR: androgen receptor; CLD: cutaneous lymphoproliferative disease; CRISPR: clustered regularly interspaced short palindromic repeats; CSR: class switch recombination; DDR: DNA damage response; DSB: double strand break; IG: immunoglobulin; NPM: nucleophosmin; RAG: recombination activating gene; SHM: somatic hypermutation; ssDNA: single stranded DNA; TALEN: transcription activator-like effector nucleases

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