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Reciprocal products of chromosomal translocations in human cancer pathogenesis: key players or innocent bystanders?

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Chromosomal translocations are frequently involved in the pathogenesis of leukemias, lymphomas and sarcomas. They can lead to aberrant expression of oncogenes or the generation of chimeric proteins. Classically, one of the products is thought to be oncogenic. For example, in acute promyelocytic leukaemia (APL), reciprocal chromosomal translocations involving the retinoic acid receptor α (*RARα*) gene lead to the formation of two fusion genes: *X–RARα* and *RARα–X* (where X is the alternative *RARα* fusion partner: *PML*, *PLZF*, *NPM*, *NuMA* and *STAT 5b*). The X–RARα fusion protein is indeed oncogenic. However, recent data indicate that the *RARα–X* product is also critical in determining the biological features of this leukemia. Here, we review the current knowledge on the role of reciprocal products in cancer pathogenesis, and highlight how their expression might impact on the biology of their respective tumour types.

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Recurring chromosomal abnormalities have been identified in a variety of cancers, but are most frequently associated with leukaemias, lymphomas and sarcomas [1,2]. At present, more than 500 recurring cytogenetic abnormalities have been reported in hematological malignancies, a frequency several times higher than that reported in mesenchymal and epithelial cancers, according to the Cancer Genome Anatomy Project/Cancer Chromosome Aberration Project of the National Cancer Institute (http://cgap.nci.nih.gov/Chromosomes/Recurrent Aberrations). Whether the observed differences in frequency of associated chromosomal abnormalities in hematological and non-hematological cancers are related to distinct mechanisms governing genomic plasticity (e.g. VDJ recombination in lymphoid cells) and/or stability in different tissues/cells, or, merely reflect technical limitations in their detection in solid tumours is still unclear.

Three main cytogenetic changes have been detected in cancer cells: chromosomal deletions, inversions

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and translocations, with translocations being by far the most frequent [1,2]. In lymphoid leukaemias and lymphomas, chromosomal translocations frequently lead to the transcriptional activation of proto-oncogenes by bringing their coding regions in the vicinity of immunoglobulin or T-cell receptor gene-regulatory elements, thus leading to their inappropriate expression [3]. Chromosomal translocations associated with Burkitt's lymphoma represent a typical example of this type of mechanism. Approximately 90% of Burkitt's lymphoma cases harbor t(8;14)(q24;q32), which juxtaposes the *MYC* proto-oncogene to the immunoglobulin heavy-chain gene (IgH) [3]. In addition, the variant translocations t(2;8) and t(8;22) described in this disease also involve *MYC*, which translocates into the immunoglobulin κ and λ light-chain loci, respectively. Structurally, the coding region of *MYC* is not altered, suggesting that its oncogenic activity is a result of its mis- or overexpression, although rare somatic mutations in the coding region of *MYC* have also been described in Burkitt's lymphoma [4,5]. Indeed, the relationship between *MYC* transcriptional activation and oncogenesis was demonstrated by the generation of transgenic mice (TM) in which the *MYC* gene was expressed under the control of the IgH enhancer. Eμ-myc TM developed clonal B lymphoid malignancies, after a long prelymphomatous phase characterized by the expansion of polyclonal pre-B cells [6].

Aberrant expression of the *BCL-6* proto-oncogene caused by chromosomal translocations is also frequently reported in non-Hodgkin's lymphoma. *BCL-6* is normally expressed in B and $CD4^+$ T cells in germinal centers (GC), and plays an essential role in GC formation [7]. *BCL-6* expression is deregulated in non-Hodgkin lymphomas arising from GC B cells, as a result of two distinct mechanisms: (1) chromosomal translocations leading to the substitution of the gene promoter region by sequences of a partner gene [8]; and (2) mutations affecting its 5′ noncoding regulatory region [9].

In addition to proto-oncogene transcriptional activation, chromosomal translocations might cause gene fusions, as frequently observed in human leukaemias. In this case, the translocation splits the genes on both partner chromosomes leading to the juxtaposition of part of each gene. The resulting fusion gene often encodes a chimeric protein [1,2]. This is probably the most common genetic abnormality associated with human cancer (see Tables 1–3 for the most frequent chromosome abnormalities causing gene fusions associated with myeloid, lymphoid and solid malignancies, respectively). Many of the breakpoints involved in specific chromosomal translocations have been cloned, and, in some cases, the molecular mechanisms leading to tumourigenesis partially elucidated. It should be noted that the genes involved in these fusions frequently encode transcription factors, suggesting that aberrant transcription plays a key role in oncogenesis, at least in these tumour types.

Chromosomal translocations are typically reciprocal, therefore leading to the generation of two chimeric genes. Usually, only one of the products is considered to be essential to the oncogenic process based on the fact that (1) it is detected in the majority of the patients, in contrast with the reciprocal product; (2) it retains most of the functional domains of the parental proteins; or (3) rare complex chromosomal translocations have been identified in which one of the reciprocal products is not formed, nevertheless the tumour/leukaemia exhibit the same phenotype. The third point is particularly misleading in view of the fact that in these cases, additional genetic events might functionally complement the missing oncogenic activity normally contributed by the reciprocal fusion protein.

However, in many chromosomal translocations, one of the two fusion transcripts is never detected, thus suggesting that the expression of the reciprocal product is probably not required for tumourigenesis in many cases (see Tables 1–3). Important exceptions are patients with t(9;22)/chronic myelogenous leukaemia (CML), in which the reciprocal product *ABL–BCR* is detected in ~60% of the cases [11], and patients with acute promyelocytic leukaemia (APL), in which both products are detected in the vast majority of cases [14,15]. Although for CML there is no direct experimental evidence that the reciprocal product plays a role in leukaemogenesis, recent data obtained in TM models of APL suggest that the reciprocal product might potentiate and/or modify the leukaemogenic activity of the APL-associated fusion proteins PML–RARα and PLZF–RARα. In solid tumours, such as sarcomas, the reciprocal product might also play an important role in determining the occurrence of tumours with distinct histopathological features.

Here, we discuss the role and relevance of translocation-generated reciprocal fusion products in three specific tumour types: APL, CML and alveolar soft part sarcoma (ASPS).

Reciprocal products potentiate and/or modify leukaemogenesis induced by X–RARα fusion proteins in APL. APL is a distinct subtype of acute myelogenous leukaemia (the M3 and M3-Variant subtypes according to the French–American–British classification of the acute leukaemias [59]) characterized by unique biological features, including (1) the expansion of leukaemic cells blocked at the promyelocytic stage of the myelopoiesis; (2) its invariable association with reciprocal chromosomal translocations involving the retinoic acid receptor α (*RARα*) gene on chromosome 17; and (3) its exquisite sensitivity to the differentiating action of all-*trans* retinoic acid (ATRA) [60]. At the molecular level, the *RARα* gene is fused to the promyelocytic leukaemia gene (*PML*), to the promyelocytic leukaemia zinc finger (*PLZF*) gene, to the nucleophosmin (*NPM*) gene, to the nuclear mitotic apparatus (*NuMA*) gene, or to the signal

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Table 1. Frequent gene fusions caused by chromosomal abnormalities associated with malignant myeloid diseases

Chromosome abnormality	Disease	Frequency[a]	Fusion gene	Reciprocal product[b]	Refs
t(9;22)(q34;q11.2)	CML	~98%	*BCR–ABL*	~60%	[10–11]
t(8;21)(q22;q22)	AML-M2	18% (30%)	*AML1–ETO*	Not expressed	[12,13]
t(15;17)(q21-q11-22)	AML-M3	10% (98%)	*PML–RARα*	~70%	[14]
t(11;17)(q23,q21)	AML-M3	Rare	*PLZF–RARα*	>90% cases	[15]
Inv(16) or t(16;16)	AML-M4Eo	8% (~100%)	*CBFβ–MYH11*	NA	[16]
t(9;11)(p22;q23)	AML-M4	11% (30%)	*MLL–AF9*	Inconsistently detected	[17]
t(6;11)	AML-M5	11q23 abnormalities are detected in ~35% of all AML-M5	*MLL–AF6/AF6q21*	Inconsistently detected	[17–22]
t(10;11)			*MLL–AF10; CALM–AF10*		
t(11;17)			*MLL–AF17/AF17q25*		
t(11;19)			*MLL–ENL/ENL/EEN*		
t(4;11)			*MLL–AF4*		
t(6;9)(p23; q34)	AML–M1, M2, M4, M5	1%	*DEK–CAN*	Not expressed	[23]
t(16;21)(p11;q22)	AML	<1%	*TLS(FUS)–ERG*	Inconsistently detected	[24]
t(16;21)(q24;q22)	t-AML, MDS	<1%	*AML1–MTG16*	Inconsistently detected	[25]
t(3;21)	AML	<1%	*AML1–EVI1*	Inconsistently detected	[26,27]
			AML1–EAP		
			AML1–MDS1		
t(7;11)(p15;p15)	AML-M2, M4	<1%	*NUP98–HOXA9*	Inconsistently detected	[28,29]
t(1;11)(q23;p15)	AML-M2		*NUP98–PMX1*		
t(8;16)(p11;p13)	AML-M4, M5	<1%	*MOZ–CBP*	Inconsistently detected	[30]
Inv(8)(p11,q13)	AML-M0, M1, M5	<1%	*MOZ–TIF2*	NA	[31]
t(8;22)(p11;p13)	AML-M5	<1%	*MOZ–p300*	Inconsistently detected	[32]
t(12;22)(p13;q23)	AML-M4, CML	<1%	*TEL–MN1*	Inconsistently detected	[33]
t(5;12)(q33;p12)	CMMoL	2–5%	*TEL– PDGFRβ*	Not expressed	[34]
t(1;19)(q23;p13)	AML-M7	<1%	*OTT–MAL*	Not expressed	[35]

[a]The percentage refers to the frequency of the translocations within the disease overall. The values within parentheses refer to the frequency within the morphologic or immunological subtype of the disease.
[b]The percentage refers to the frequency of the reciprocal product within cases harboring the chromosomal translocation.
Abbreviations: AML, acute myelogenous leukaemia; CML, chronic myelogenous leukaemia; CMMoL, chronic myelomonocytic leukaemia; MDS, myelodysplastic syndrome; NA, not applicable; t-AML, therapy-related AML.

transducer and activator of transcription 5B (*STAT 5B*) gene (for brevity hereafter referred to as X genes) located on chromosome 15, 11, 5, 11, and 17, respectively. These chromosomal translocations are reciprocal (Fig. 1), thus leading to the generation of *X–RARα* and *RARα–X* fusion genes and the co-expression of their chimeric products in the leukaemic blasts [60].

Structurally, the X–RARα fusion proteins retain most of the RARα functional domains (domains B–F), including the DNA- and ligand-binding domains (Fig. 1). These are linked by the C-terminal to five different moieties from the various X proteins. These various N-terminal regions, although structurally distinct, normally mediate self-association of the various X protein and, in turn, heterodimerization between X and X–RARα proteins. Unlike RARα, which can activate transcription at physiological dose of retinoic acid, the various X–RARα fusion proteins function as aberrant and dominant transcriptional repressors through heterodimerization with retinoid–*X* receptor (RXR), and at least in part, through their ability to form repressive complexes with nuclear receptor co-repressors, such as Sin3A or Sin3B and histone deacetylases (HDACs) [61]. In addition, the various X–RARα fusion proteins can interfere with their respective X pathways, through physical interaction.

Much has been learned about APL pathogenesis and the role of the X–RARα and RARα–X fusion proteins through the analysis of TM models. We, and others, have generated TM in which the *PML–RARα* fusion gene is expressed under the control of the human cathepsin G (*hCG*) or *MRP8* promoter [62–64]. After a long latency (ranging between six months to one year), 10–15% of these TM develop a lethal form of leukaemia that closely resembles human APL, the leukaemic population consisting mainly of myeloid blasts and promyelocytes. The low frequency of leukemia in these *PML–RARα* models is very likely not a result of low level or inappropriate expression of the transgene as suggested by several lines of evidence such as (1) the analysis of β-galactosidase expression in myeloid progenitors from *hCG–Lac-Z* TM, which demonstrates that the cathepsin G minigene expression vector retains the elements that are necessary and sufficient for promyelocytic specific transgene expression [63]; (2) the fact that the *MRP8* promoter drives very high levels of transgene expression in the myeloid cellular compartment [64]; (3) the generation of knock-in mutants (i.e. *X–RARα* gene inserted in the *X* loci) in which leukemia onset

Table 2. Frequent gene fusions caused by chromosomal abnormalities associated with malignant lymphoid diseases

Chromosome abnormality	Disease	Frequency[a]	Fusion gene	Reciprocal product[b]	Refs
t(1;19)(q23;p13)	Pre-B ALL	6% (30%)	*E2A–PBX1*	Not expressed	[36]
t(17;19)(q22;p13)	Pro-B ALL	1%	*E2A–HLF*	Not expressed	[37]
t(12;21)(p12-13;q22)	Pre-B ALL	25%	*TEL–AML1*	Inconsistently detected	[38,39]
t(9;22)(q34;q11.2)	ALL	~5% of childhood ALL; 30% of adult B-ALL	*BCR–ABL*	~60%	[40]
t(4;11)(q21;q23)	Pre-B ALL	5%	*MLL–AF4*	Inconsistently detected	[17,18,21, 41,42]
t(9;11)(p22;q23)	Pre-B ALL	<1%	*MLL–AF9*		
t(11;19)(q23;p13)	Pre-B ALL, T-ALL	1%	*MLL–ENL*		
t(X;11)(q13;q23)	T-ALL	<1%	*MLL–AFX1*		
t(1;11)(p32;q23)	ALL	<1%	*MLL–AFP1*		
t(6;11)(q27;q23)	ALL	<1%	*MLL–AF6*		
inv(2;2)(p13;p11-14)	NHL	<1%	*REL/NGR*	NA	[43]
t(2;5)(2p23; q35)	Lymphoma ALCL	75%	*NPM–ALK*	Not expressed	[44]
t(3;14)	DLCL	~30%	*IG–BCL6*	Not expressed	[45]
	follicular lymphomas	~10%			
t(4;16)	T-lymphoma	<1%	*BCM–IL2*	Not expressed	[46]

[a]The percentage refers to the frequency of the translocations within the disease overall. The values within parentheses refer to the frequency within the morphological or immunological subtype of the disease.
[b]The percentage refers to the frequency of the reciprocal product within cases harboring the chromosomal translocation.
Abbreviations: ALCL, anaplastic large cell lymphoma; ALL, acute lymphoblastic leukaemia; DLCL, diffuse large B-cell lymphomas; NA, not applicable; NHL, non-Hodgkin lymphoma;.

and frequency are not accelerated; (4) the fact that leukaemia in *hCG–PLZF–RARα* TM is completely penetrant (see below).

As in human APL, *PML–RARα* TM leukaemia responds to RA treatment that induces the terminal differentiation of the leukaemic blasts. Human APL patients harboring t(11;17) (*PLZF–RARα*), however, unlike other APL patients, do not respond to ATRA. We generated *hCG–PLZF–RARα* TM as a model of t(11;17)/APL [65]. In contrast to leukaemia in *hCG–PML–RARα* TM, leukaemia in *hCG–PLZF–RARα* TM lacks the distinctive block of differentiation at the promyelocytic stage of myelopoiesis [65] and is characterized by leukocytosis and infiltration of all organs by terminally differentiated myeloid cells (a situation that resembles human chronic myeloid leukaemia). As in *hCG–PML–RARα* TM, this leukaemia develops after a long latency of approximately six months. However, the leukaemic phenotype is now completely penetrant [65]. Moreover, *hCG–PLZF–RARα* TM do not respond to RA treatment.

All together, these results demonstrate that (1) X–RARα proteins are necessary but not sufficient to cause APL leukaemogenesis, as supported by the fact that leukaemia occurs, but only after a long latency and that *hCG–PLZF–RARα* TM do not develop a classic APL phenotype; (2) the differential

Table 3. Frequent gene fusions caused by chromosomal abnormalities associated with malignant solid tumours

Chromosome abnormality	Disease	Fusion gene	Reciprocal product	Ref
t(11;22)(q24;q12)	Ewing's sarcoma	*EWS–FL1*	Not expressed	[47]
t(21;22)(q22;q12)	Ewing's sarcoma	*EWS–ERG*	Not expressed	[48]
t(7;22)(p22;q12)	Ewing's sarcoma	*EWS–ETV1*	Not expressed	[49]
t(12;22)(q13;q12)	Clear cell sarcoma	*EWS–ATF1*	Not expressed	[50]
t(9;22)(q22-31;q12)	Myxoid chondrosarcoma	*EWS–CHN*	Not expressed	[51]
t(11;22)(p13;q12)	Desmoplastic small round cell tumour	*EWS–WT1*	Not expressed	[52]
inv 10(q11.2;q21)	Papillary thyroid carcinoma	*RET–PTC1/PTC3*	NA	[54]
t(10;17)		*RET–PTC2*		
t(X;18)(p11.2;q11.2)	Synovial sarcoma	*SYT–SSX1/SSX2*	Not expressed	[55]
t(2;13)(q37;q14)	Alveolar rhabdomyosarcoma	*PAX3–FKHR*	Not expressed	[56]
t(1;13)(q36;q14)		*PAX7–FKHR*		
Der(17)t(X;17)(p11.2;q25)	Alveolar soft part sarcoma	*ASPL–TFE3*	Not expressed[a]	[57]
t(X;17)(p11.2;q25)	Papillary renal adenocarcinomas	*ASPL–TFE3*	Expressed	
t(12;16)(q13;p11)	Myxoid/round cell liposarcomas	*TLS/FUS–CHOP*	Not determined	[58]

[a]The balanced and unbalanced forms of the t(X;17) are associated with distinct tumours (see text for details).
Abbreviation: NA, not applicable.

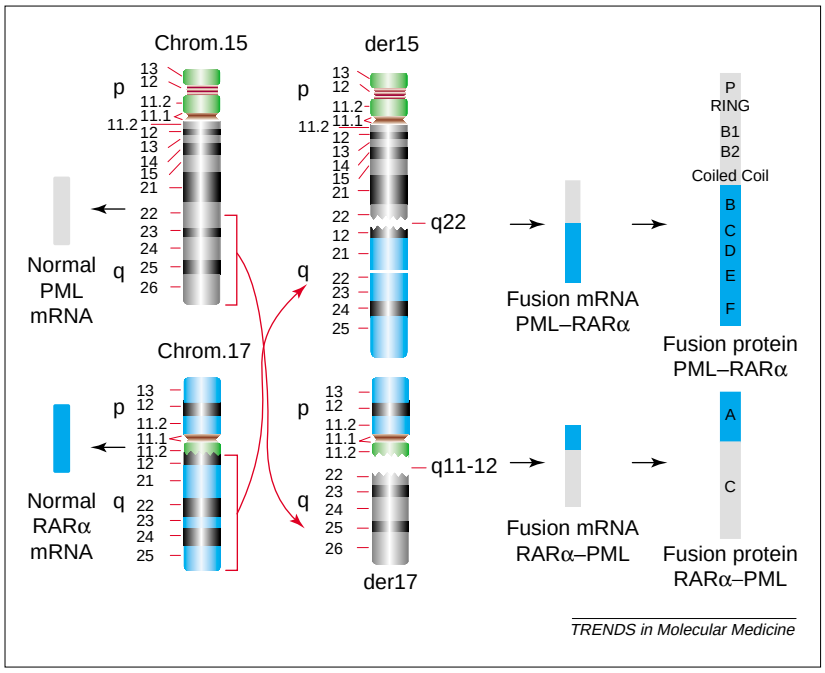

Fig. 1. Reciprocal translocations between chromosomes 15 and 17 involving the *PML* and the *RARα* genes generate *PML–RARα* and *RARα–PML* fusion genes. The various functional domains from the PML (in grey) and RARα (in blue) proteins are indicated: P, proline rich domain; RING (really interesting gene) domain, a C_3HC_4 zinc-finger domain; B1, B2, B boxes, two additional zinc fingers; coiled-coil region; C, C-terminal end. A, B, transactivation domains; C, DNA binding domain; D, hinge domain; E: ligand binding domain; F, transcriptional regulatory region. The t(15;17) is the most common chromosomal aberration associated with APL. Variant chromosomal translocations invariably involving the same *RARα* intron have also been described in APL, which are always reciprocal and balanced.

response to RA observed between t(11;17) and t(15;17)APL is mediated by the X–RARα fusion proteins.

The RARα–X fusion proteins could therefore represent a possible cooperating event toward full-blown APL leukaemogenesis. Pollock *et al.* [66] studied the role of *RARα–PML*, by generating *hCG–RARα–PML* TM. No alteration in myeloid development or overt leukaemia was detected in this TM model, although the reciprocal *RARα–PML* was expressed at high levels. However, *RARα–PML* increased the frequency of APL in double *PML–RARα/RARα–PML* TM (Fig. 2). However, these leukaemias respond *in vitro* to RA in similar manner. Given that the *RARα–PML* cDNA used by Pollock *et al.* [66] encodes a fusion protein that retains PML B-boxes, heterodimerization interface (coiled-coil region) and its C-terminal domain [66] (Fig. 1), it is conceivable that the RARα–PML activity is mediated by deregulation of PML protein–protein interactions. In fact, PML can interact with the nonphosphorylated fraction of retinoblastoma (Rb) and p53 tumour suppressors via its C-terminal domains [67,68]. Consistent with this notion, we have recently demonstrated that *Pml* inactivation leads to an increase in frequency and acceleration of leukaemia onset in *hCG–PML–RARα/Pml*$^{-/-}$ mutants [69].

In contrast to RARα–PML, which does not appear to bind DNA directly, RARα–PLZF retains seven out of nine Krüppel-type zinc fingers that constitute the PLZF DNA-binding domain [70]. RARα–PLZF is also able to interact with PLZF DNA-responsive elements, but lacks the transcriptional repressive ability of PLZF [71]. This is a result of the fact that the N-terminal transcriptional repression POZ domain of PLZF is replaced in RARα–PLZF by one of the transacting domains from RARα (A domain). In addition, RARα–PLZF is able to homodimerize with PLZF through its zinc fingers.

RARα–PLZF TM do not develop overt leukaemia, but display aberrant hematopoiesis characterized by a slow and progressive accumulation of myeloid cells in BM and spleen (myeloid hyperplasia), which does not affect peripheral blood counts. After the first year of life, ~20% of the *RARα–PLZF* TM develops this syndrome [71]. In contrast to *hCG–PLZF–RARα*, *PLZF–RARα/RARα–PLZF* double TM develop leukaemia with classical APL features, now characterized by the expansion of immature promyelocytes (Fig. 3). Furthermore, the expression of *RARα–PLZF* in *hCG–PLZF–RARα* leukaemic cells results in (1) increased proliferation rates, as evaluated by thymidine incorporation assays; (2) increased numbers of leukaemic clusters in a methylcellulose assay; and (3) reduced rate of apoptosis [71]. Therefore, *RARα–PLZF* expression confers proliferative and survival advantage to the *PLZF–RARα* leukaemic cell. Strikingly, *RARα–PLZF* further exacerbates RA unresponsiveness of *PLZF–RARα* leukaemic cells *in vitro* [71]. Thus, RARα–PLZF affects both the biology of the disease and its response to therapy.

As mentioned above, RARα–PLZF can act as a dominant-negative inhibitor of PLZF function. We validated this hypothesis *in vivo* by intercrossing *hCG–PLZF–RARα* TM with mice in which we have inactivated *Plzf* by homologous recombination (*Plzf*$^{-/-}$ mice) [71,72]. *Plzf*$^{-/-}$ mice display important skeletal abnormalities, but do not present overt defects in myelopoiesis [72]. However, *hCG–PLZF–RARα/Plzf*$^{-/-}$ mutants develop a form of leukaemia morphologically and immunophenotypically indistinguishable from the leukaemia observed in *RARα–PLZF/PLZF–RARα* double TM [71]. Surprisingly, the co-expression *RARα–PLZF* did not affect leukaemia onset in *hCG–PLZF–RARα* TM, which develop leukaemia approximately after a six-month latency.

All together, these results unravel the importance of the reciprocal *RARα–X* product in APL pathogenesis and suggest that these molecules might play an important role in the deregulation of the X pathways. While *RARα–PML* seems to act as a classical tumour modifier affecting leukaemia onset, *RARα–PLZF* metamorphoses the CML-like phenotype observed in *hCG–PLZF–RARα* TM in classical APL. The essential role of *RARα–PLZF* in dictating the APL phenotype is supported by the fact that only one out of 11 t(11;17)/APL cases analyzed did not express the reciprocal product [15]. In this case, the key function

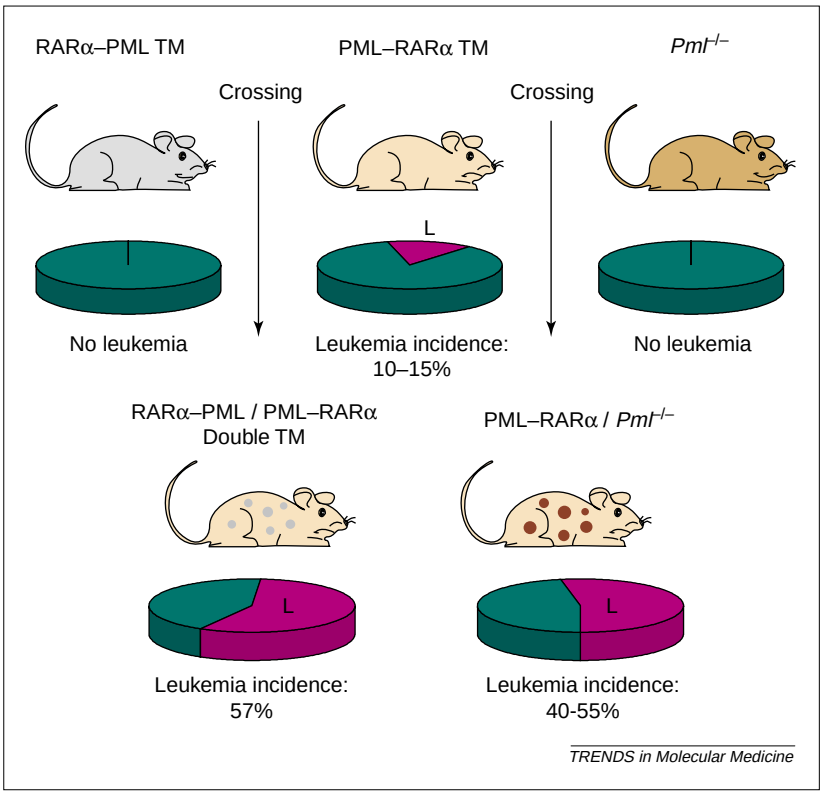

Fig. 2. Expression of *RARα–PML* or inactivation of *Pml* increases the frequency of leukaemia (L) in *PML–RARα* transgenic mice (TM). *PML–RARα* TM develop a form of leukaemia that closely resembles human APL. The disease occurs in ~10–15% of the TM, after a long (~12 months) pre-leukaemic phase. *RARα–PML* TM and mice in which the *Pml* gene was inactivated by homologous recombination (*Pml*$^{-/-}$ mice) do not develop leukemia. Double TM *PML–RARα/RARα–PML PML–RARα/Pml*$^{-/-}$ mutants were obtained by crossing *PML–RARα* TM with *RARα–PML* TM and *Pml*$^{-/-}$ mutants, respectively. The expression of *RARα–PML* or the inactivation of *Pml* resulted in an increase of leukemia incidence and an acceleration of its onset.

of RARα–PLZF might be replayed by additional unidentified genetic events (e.g. inactivation of PLZF function).

In t(15;17)/APL, the expression of reciprocal product *RARα–PML* did not influence the survival rate, morphological subtype (M3 *v* M3V) or presence of coagulopathy [73,74,]. Nevertheless, support for an important role of RARα–PML in leukemia pathogenesis came from the analysis of rare APL cases lacking the t(15;17), in which the fusion genes were generated by submicroscopic insertions of *RARα* or *PML* into chromosomes 15 or 17, respectively, thus resulting in the expression of either product [75]. Leukaemic cells solely harboring *RARα–PML* or *PML–RARα* showed the same morphological features, but distinct pattern of immunostaining for PML and response to ATRA *in vitro*, the former exhibiting resistance to the treatment [75].

Leukemia in *hCG–PML–RARα* and *hCG–PLZF–RARα/RARα–PLZF* double TM resemble human APL because of (1) the clonal expansion of cells blocked at the promyelocytic stage of myelopoiesis; and (2) response or resistance to ATRA treatment. However, leukemia in TM is preceded by a long pre-leukaemic phase, characterized as myeloproliferative disorder that is not observed in human APL (six months on average in both *PML–RARα/RARα–PML* and *PLZF–RARα /RARα–PLZF* models [66,71]), suggesting that additional genetic events might still be necessary for the development of full-blown leukemia in mice. This is supported by the finding that double TM accumulate recurrent chromosomal abnormalities throughout disease evolution [76,77]. Chromosomal abnormalities involving syntenic regions are also observed in human APL, thus making these transgenic models a unique tool to characterize these genetic lesions at the molecular level. Nevertheless, it should be emphasized that differences between these transgenic models and the human disease might hamper the interpretation of the results. For instance, the relative level of expression of the reciprocal products might differ in the blasts from APL patients and leukaemic mice. Moreover, it should pointed out that as a result of the chromosomal translocation in humans the normal X and *RARα* genes are reduced to hemizygosity, whereas the TM still harbor two normal copies of the parental genes.

The reciprocal fusion product ABL–BCR is frequently expressed in CML and retains the BCR domain with GTPase-activating function

CML is a malignant myeloproliferative disease characterized by the hyperplasia of well differentiated myeloid cells and by its association with chromosomal translocation between chromosomes 9 and 22 [t(9;22)], which generates the Philadelphia (Ph) chromosome [10,78]. This translocation creates two chimeric genes: the *BCR–ABL* on the derivative chromosome 22, and the *ABL–BCR* on the derivative chromosome 9 [10,11,78]. The t(9;22) is the hallmark of CML. In 98% of CML patients it can be detected by classic cytogenetic techniques, fluorescent *in situ* hybridization (FISH) or reverse transcriptase-polymerase chain reaction (RT-PCR). Rarely, a third chromosome is involved in addition to chromosomes 9 and 22 in complex translocations [78].

The BCR–ABL fusion protein exhibits deregulated protein tyrosine kinase (PTK) activity exerted through its ABL moiety, which results in excessive phosphorylation of several intracellular proteins, including BCR–ABL itself. BCR–ABL is localized in the cytoplasm and its PTK activity is essential for malignant transformation [79]. Multiple substrates of BCR–ABL have been identified, such as the CDK2 serine/threonine kinase, crk like proteins (CRKL), p62^{DOK} and growth receptor binding protein-2 (GRB2) [80]. As a result, the expression of BCR–ABL affects multiple pathways, including the RAS, phosphatidylinositol 3′kinase (PtdIns-3kinase), nuclear factor (NF)-κB, Janus family kinases (JAK) and the signal transducer and activation of transcription (STAT) pathways [81]. BCR–ABL exerts critical leukaemogenic functions such as (1) induction of growth factor independence;

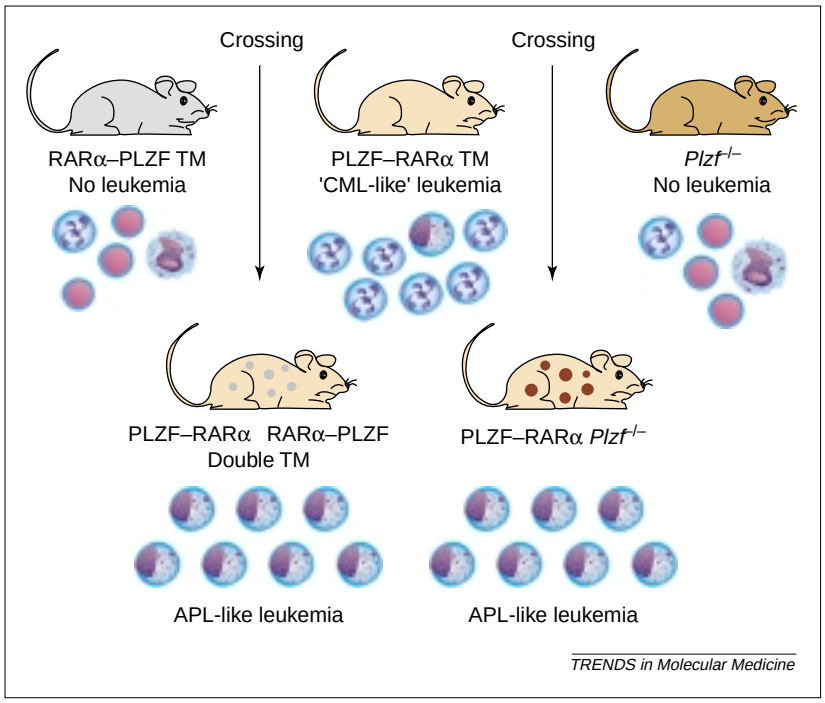

Fig. 3. Expression of *RARα–PLZF* or inactivation of *Plzf* metamorphose the leukaemic phenotype in *PLZF–RARα* transgenic mice (TM). *PLZF–RARα* TM develop a form of leukaemia characterized by the expansion of mature myeloid cells. *RARα–PLZF* TM and mice in which the *Plzf* gene was inactivated by homologous recombination (*Plzf*$^{-/-}$ mice) do not develop overt leukemia. Double TM *PLZF–RARα /RARα–PLZF* and *PLZF–RARα/Plzf*$^{-/-}$ mutants were obtained by crossing *PLZF–RARα* TM with *RARα–PLZF* TM and *Plzf*$^{-/-}$ mutants, respectively. The expression of *RARα–PLZF* or the inactivation of *Plzf* metamorphosed the leukaemia phenotype in *PLZF–RARα* TM, now characterized by the expansion of immature myeloid cells blocked at the promyelocytic stage as observed in human APL.

(2) inhibition of apoptosis; and (3) inhibition of the adhesion of chronic myeloid progenitor cells to bone marrow stroma, which in turn can facilitate leukaemia dissemination [79–81]. Thus, there is little doubt that BCR–ABL plays a crucial role in CML pathogenesis. However, the reciprocal product *ABL–BCR* is also detected in ~60% of CML patients [11,82,83], both in chronic phase as well as in blast crisis. Moreover, in *BCR–ABL*-positive human leukaemia cell lines, *ABL–BCR* transcripts were detected in 56% and 66% of the lymphoid and myeloid cell lines, respectively [84]. Two possible splicing variants of the *ABL–BCR* fusion gene have been described: *ABL1a–BCR* and *ABL1b-BCR*, containing *ABL* exon 1a or 1b, respectively. *ABL1b–BCR* is preferentially expressed both in CML patients and in *BCR–ABL*-positive cell lines [11,84].

The BCR moiety from the ABL–BCR chimeric protein retains a GTPase-activating protein (GAP) domain of BCR, which can regulate the activity of $p21^{rac}$, a member of the RAS family of small GTP-binding proteins, which is involved in the regulation of the actin cytoskeleton [85]. The rearrangement of the GAP domain of BCR can lead, in principle, either to constitutive activation or inactivation of the racGAP activity, and it is therefore conceivable that the reciprocal product of the t(9;22) might act as an additional mutagenic event for CML oncogenesis. Unfortunately, to date, no animal model in which both reciprocal products are expressed has been generated. This would be essential to analyze the biological consequences of *ABL–BCR* expression on phenotype, time of onset and leukaemia progression.

Regarding treatment, *ABL–BCR* expression does not affect CML response to interferon α [82,83], while no studies have been so far conducted to correlate *ABL–BCR* expression with response to STI571 treatment. Furthermore, Fuente *et al.* [83] have recently demonstrated that *ABL–BCR* expression does not correlate with the presence of large deletions on the 9q+ derivative (a powerful negative prognostic feature), or with a shorter survival in CML patients.

Balanced or unbalanced forms of the t(X;17)(p11;q25) generating *ASPL and TFE3* fusion genes are associated with distinct tumour types

Alveolar soft part sarcoma (ASPS) is a rare tumour that usually arises in the soft tissues of the upper and lower limbs [86]. It characteristically affects young adults (peak incidence age of between 15 and 35 years) and the majority of patients present advanced-stage disease. The disease has a relatively indolent clinical course but long-term mortality is high because of chemoresistance of metastatic disease [86]. ASPL has distinctive histopathological features: these tumours are made of oval or polyhedral cells, distributed in pseudoalveolar arrangement that contain characteristic cytoplasmic crystals [87]. Cytogenetically, the tumour is specifically associated with the non-reciprocal translocation der(17)t(X;17)(p11.2;q25), which fuses the *TFE3* gene (which encodes for a basic helix–loop–helix family member transcription factor) on the X chromosome to the *ASPL* gene on chromosome 17, whose function is presently unknown [57,88]. Two alternative types of *ASPL–TFE3* fusion products can be generated as a consequence of t(X;17): type 1, in which the *TEF3* exon 3 is excluded from the transcript, and type 2, which retains this region. The ASPL–TFE3 fusion proteins encoded by these fusion genes can function as aberrant transcription factors (M.Y. Lui, M. Ladanyi, pers. commun.), as also demonstrated for the PRCC–TFE3 fusion protein, generated by the t(X,1) in a subset of papillary renal adenocarcinomas and involving the same TFE3 domains [89].

Remarkably, the same *ASPL–TFE3* fusion gene is also produced by a reciprocal t(X;17)(p11.2;q25) in a distinct subset of papillary renal adenocarcinomas [57] (Fig. 4). These renal tumours occur earlier in life than ASPS and show a nested and pseudopapillary pattern of growth, psammomatous calcifications, and epithelioid cells with abundant clear cytoplasm. Furthermore, electron microscopy and immunohistochemical studies demonstrate epithelial features, at least focally, in most cases. However, the cytoplasmic crystals typical of ASPS are present in some of these renal tumours as well, thus demonstrating that these unusual and unique renal

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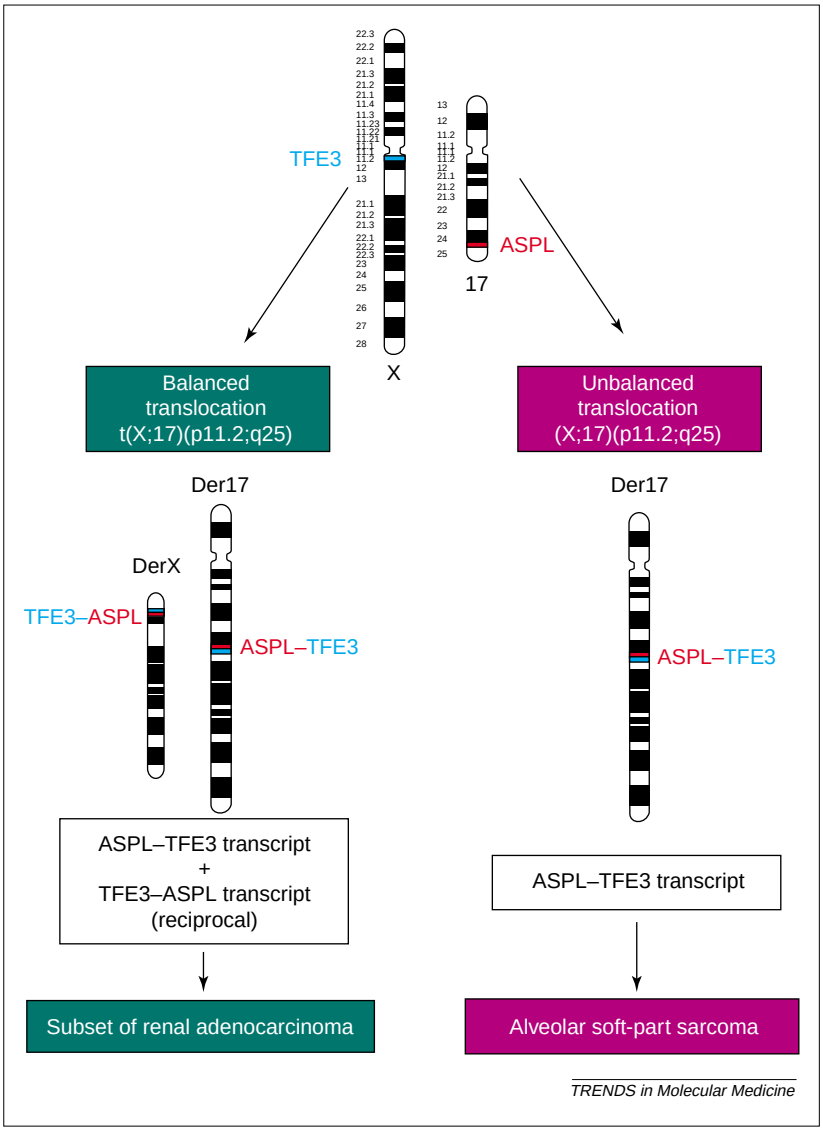

Fig. 4. Balanced and unbalanced chromosomal translocations between chromosomes X and 17 involving the *ASPL* and the *TFE3* genes are associated with distinct primary renal tumours.

tumours have features of both ASPS and renal adenocarcinoma. Out of eight *ASPL–TFE3* fusion-positive renal tumours that harbored the balanced translocation between chromosomes X and 17 (confirmed by fluorescence *in situ* hybridization in seven cases), five expressed the reciprocal *TFE3–ASPL* fusion transcript [57]. In four of these five cases, the *TFE3* and *ASPL* genes were fused in-frame in the reciprocal transcripts and therefore capable of encoding functional fusion proteins. Studies examining the level of protein expression of this *TFE3–ASPL* product and its activity in transactivation assays are in progress. Hypothetically, the marked clinical and phenotypic differences between ASPS and these renal tumours might be a result of the aberrant activity of the reciprocal *TFE3–ASPL* product in the latter tumour type (in the minority of these renal tumours where this product is not expressed, additional events might compensate for its absence). In a second hypothetical scenario, the *TFE3–ASPL* product might interfere with ASPS tumourigenesis, but not with renal tumours pathogenesis, with a resulting selective pressure for an unbalanced translocation in ASPS. Finally, the selective pressure for an unbalanced translocation in ASPS might be a result of the loss of a tumour suppressor at 17q25.3->qter, which is operative in ASPS, but not in the renal tumours. These different hypotheses, which are not mutually exclusive, need to be tested in the future through *in vivo* approaches in knockout or transgenic mice.

Concluding remarks

Although a considerable number of human cancers are associated with reciprocal chromosomal translocations, the detection of the products encoded by both derivatives is infrequent. Notably, however, the reciprocal product is more often expressed in hematological malignancies. Among the leukaemias, both products are frequently detected in APL and CML. Experimental data obtained in transgenic models of APL suggest that the expression of the reciprocal product influence disease penetrance, phenotype and treatment response. By contrast, such evidence is lacking for CML. Among solid tumours, the translocation between chromosomes X and 17 represent a prototypical example of the importance of the reciprocal product. An unbalanced translocation involving *ASPL* and *TEF3* genes is associated with alveolar soft part sarcoma, whereas a balanced translocation involving the same loci, which leads to the expression of both fusion genes, is associated with a morphologically distinct subset of papillary renal adenocarcinomas. Although the detailed molecular role of the various reciprocal products remains to be elucidated, it is becoming apparent that, when expressed, these molecules often play an important role in tumourigenesis and/or in modulating response to therapy.

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