MINIREVIEW

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Functional and cancer genomics of *ASXL* family members

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Additional sex combs-like (ASXL)1, *ASXL2* and *ASXL3* are human homologues of the *Drosophila Asx* gene that are involved in the regulation or recruitment of the Polycomb-group repressor complex (PRC) and trithorax-group (trxG) activator complex. ASXL proteins consist of ASXN, ASXH, ASXM1, ASXM2 and PHD domains. ASXL1 directly interacts with BAP1, KDM1A (LSD1), NCOA1 and nuclear hormone receptors (NHRs), such as retinoic acid receptors, oestrogen receptor and androgen receptor. ASXL family members are epigenetic scaffolding proteins that assemble epigenetic regulators and transcription factors to specific genomic loci with histone modifications. ASXL1 is involved in transcriptional repression through an interaction with PRC2 and also contributes to transcriptional regulation through interactions with BAP1 and/or NHR complexes. Germ-line mutations of human *ASXL1* and *ASXL3* occur in Bohring-Opitz and related syndromes. Amplification and overexpression of *ASXL1* occur in cervical cancer. Truncation mutations of *ASXL1* occur in colorectal cancers with microsatellite instability (MSI), malignant myeloid diseases, chronic lymphocytic leukaemia, head and neck squamous cell carcinoma, and liver, prostate and breast cancers; those of *ASXL2* occur in prostate cancer, pancreatic cancer and breast cancer and those of *ASXL3* are observed in melanoma. *EPC1-ASXL2* gene fusion occurs in adult T-cell leukaemia/lymphoma. The prognosis of myeloid malignancies with misregulating truncation mutations of ASXL1 is poor. ASXL family members are assumed to be tumour suppressive or oncogenic in a context-dependent manner.

The *Additional sex combs (Asx)-like 1 (ASXL1)* gene (Fisher *et al*, 2003), *ASXL2* gene (Katoh and Katoh, 2003) and *ASXL3* gene (Katoh and Katoh, 2004) are human homologues of the *Drosophila Asx* gene, which was initially identified based on a mutant phenotype (appearance of ectopic sex combs on the legs) and then characterised as one of *Polycomb group (PcG)* genes that are required for long-term repression of homoeotic genes (Jürgens, 1985; Breen and Duncan, 1986; Simon *et al*, 1992; Sinclair *et al*, 1992, 1998).

Drosophila PcG genes can be divided into classes based on their genetic and biochemical interactions (Simon *et al*, 1993; Cheng *et al*, 1994; Campbell *et al*, 1995; Brock and van Lohuizen, 2001; Grimaud *et al*, 2006; Sparmann and van Lohuizen, 2006; Simon and Kingston, 2009). The *Drosophila Pc, Polyhomeotic, Sex comb on midleg, Posterior sex combs* and *Sex combs extra (Ring)* genes encode core components of the Polycomb repressor complex 1 (PRC1). The *Drosophila Extra sex combs, Enhancer of zeste* and *Suppressor of zeste 12* genes encode core components of the Polycomb repressor complex 2 (PRC2). The *Drosophila Asx, Calypso, Pleiohomeotic, Pleiohomeotic-like, dSfmbt* and *Polycomb-like* genes encode cofactors or regulators of PRC1 or PRC2. PRC2 is recruited to a specific genomic loci, where it catalyses the trimethylation of histone H3 at lysine 27 (H3K27me3). Polycomb repressor complex 1 is then recruited by the H3K27me3 chromatin mark and catalyses the mono-ubiquitination of histone H2A at lysine 119, resulting in transcriptional repression of the PcG target genes (Schuettengruber *et al*, 2007; Schwartz and Pirrotta, 2007; Müller and Verrijzer, 2009). Polycomb-group repressor proteins have a key role in the maintenance of lineage-specific gene expression programs (Pietersen and van Lohuizen, 2008).

Most *Asx* mutants show posterior homoeotic transformation due to the loss of PcG functions, whereas several *Asx* mutants have been subsequently found to show not only posterior homoeotic transformations due to the loss of PcG functions but also anterior homoeotic transformations resulting from a loss of trithorax-group (trxG) functions (Milne *et al*, 1999; Brock and Fisher, 2005). Derepression of *Drosophila abdA* and *AbdB* homoeotic genes is not so significant in the central nervous system (CNS) of *Drosophila*

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Asx mutants compared with other *PcG* mutants (Simon *et al*, 1992). Derepression of *Ubx* homoeotic gene is also not significant in the CNS of most *Asx* mutants (Sinclair *et al*, 1998), whereas derepression of *Ubx* homoeotic gene occur in the wing blade of C-terminally deleted *Asx* mutant, which results in the wing defect phenotype (Bischoff *et al*, 2009). As *Drosophila* Asx has dual roles in the transcriptional regulation of homoeotic and non-homoeotic genes as a cofactor of both the PcG repressor complex and the trxG activator complex, Asx is involved in the transcriptional repression and activation of its target genes in a context-dependent manner (Sinclair *et al*, 1998; Gildea *et al*, 2000; Brock and van Lohuizen, 2001; Dietrich *et al*, 2001; Grimaud *et al*, 2006; Halachmi *et al*, 2007; Petruk *et al*, 2008).

Since the discovery of the human *ASXL* family genes in the early 2000s (Fisher *et al*, 2003; Katoh and Katoh, 2003, 2004), >130 manuscripts on ASXL1, ASXL2 or ASXL3 have been published by various research groups worldwide (Figure 1A). The number of ASXL1 manuscripts drastically increased after a report described frequent *ASXL1* mutations in human myeloid malignancies (Gelsi-Boyer *et al*, 2009). Truncation mutations of the *ASXL1* gene frequently occur in myeloid malignancies, whereas amplification, translocation or point mutations of the *ASXL1, ASXL2* or *ASXL3* genes have been observed in other types of human cancers (Table 1). *ASXL1* mutations in malignant myeloid diseases are reviewed elsewhere from a haematological point of view (Tefferi, 2010; Gelsi-Boyer *et al*, 2012). Large amounts of information on cancer genomics have been accumulated in the public database owing to innovation in nucleotide sequencing technology; however, data of cancer genomics themselves might look like printouts of 'laundry list' if not functionally annotated. Here, functional and cancer genomics of the mammalian *ASXL* family genes are reviewed as the warp and woof of ASXL tapestry, respectively, to get bird's eye view of biological functions and genetic alterations of ASXL family members in human cancers. New insights and future directions of researches on the mammalian ASXL family will be described.

ASXL FAMILY GENES

The human *ASXL* gene family consists of three members: the *ASXL1* gene is located at human chromosome 20q11.21 and is surrounded by *KIF3B, DNMT3B* and *MAPRE1* genes; the *ASXL2* gene is located at human chromosome 2p23.3 and is surrounded by *DNMT3A, DTNB, KIF3C, FAM59B* and *MAPRE3* genes; the *ASXL3* gene is located at human chromosome 18q12.1 and is surrounded by *FAM59A*, *DTNA* and *MAPRE2* genes. As the *ASXL* family genes and the *DNMT3*, *DTN*, *KIF3*, *FAM59* and *MAPRE* (sub)family genes are paralogues, the *KIF3B-ASXL1* ~ *DNMT3B-MAPRE1* locus, the *DNMT3A-DTNB-ASXL2-KIF3C* ~ *FAM59B* ~ *MAPRE3* locus and the *FAM59A* ~ *ASXL3-DTNA-MAPRE1* locus are paralogous regions within the human genome.

ASXL FAMILY PROTEINS

The human ASXL3 protein (2248 amino acids) is significantly larger than the human ASXL1 (1541 amino acids) and ASXL2 (1435 amino acids) proteins (Katoh and Katoh, 2004). The mouse and chicken Asxl3 orthologues are also significantly larger

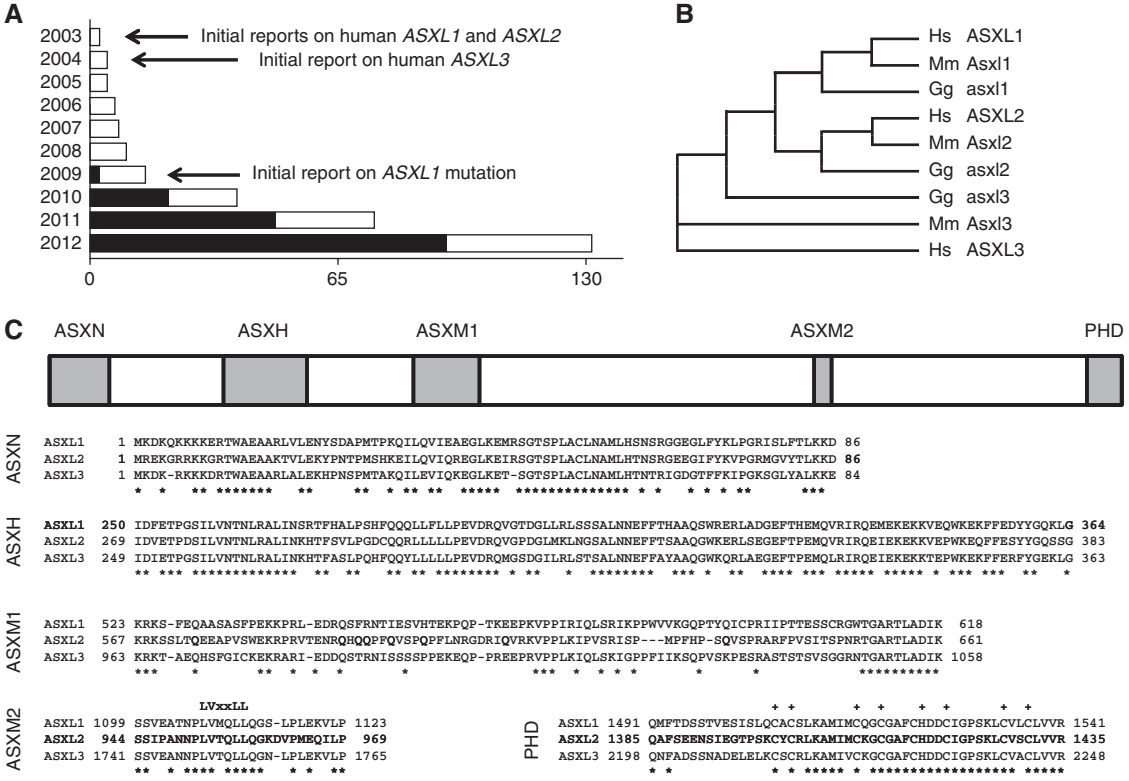

Figure 1. The ASXL family. (**A**) Chronology of the ASXL family. Cumulative publication numbers of ASXL1, ASXL2 or ASXL3 manuscripts in the PubMed database are shown by open bars, whereas those of ASXL1 mutations in malignant myeloid diseases are depicted with closed bars. (**B**) Phylogeny of the vertebrate ASXL family members. Hs, human; Gg, chicken; Mm, mouse. (**C**) Conserved domain architecture of the ASXL family members, consisting of the ASXN, ASXH, ASXM1, ASXM2 and PHD domains. The LVxxLL motif in the ASXM2 domain and the C4HC3 motif in the PHD domain are shown above the amino-acid alignment. The conserved amino acids are shown by asterisks below the alignment.

Table 1. Cancer genomics of the ASXL family genes

Gene	Genetic alterations	Cancer	Mutation rate
ASXL1	Gene amplification	Cervical cancer	4/79 (5.1%)
	Truncation mutation	CRC with MSI	6/11 (55%)[a]
		CMML	124/274 (45.3%)
		MPN	41/119 (34.5%)
		AML (secondary)	30/99 (30%)
		MDS	148/914 (16.2%)
		Liver cancer	1/10 (10%)
		AML (*de novo*)	130/2000 (6.5%)
		CLL	3/105 (2.9%)
		Prostate cancer[b]	1/50 (2.0%)
		HNSCC	1/74 (1.4%)
		Breast cancer	1/100 (1.0%)
ASXL2	Truncation mutation	Prostate cancer[b]	3/50 (6.0%)
		Pancreatic cancer	1/24 (4.2%)
		Breast cancer	1/100 (1.0%)
	Chromosomal translocation	ATLL	Rare
ASXL3	Truncation mutation	Melanoma	1/25 (4.0%)

Abbreviations: AML = acute myeloid leukaemia; ATLL = adult T-cell leukaemia/lymphoma; CLL = chronic lymphocytic leukaemia; CMML = chronic myelomonocytic leukaemia; CRC with MSI = colorectal cancer with microsatellite instability; HNSCC = head and neck squamous cell carcinoma; MDS = myelodysplastic syndrome; MPN = myeloproliferative neoplasm.
[a]Mutation rate in CRC with MSI is based on results in cell lines, whereas mutation rates in other malignancies are based on results in tumour samples.
[b]Castration-resistant prostate cancer.

than their Asxl1 and Asxl2 orthologues. Phylogenetic analyses of the human, mouse and chicken ASXL family proteins reveal that the ASXL1 and ASXL2 orthologues are more closely related (Figure 1B).

The ASXL1, ASXL2 and ASXL3 proteins share a common domain architecture that consists of an ASXN domain in the N-terminal region, an ASXH domain in the N-terminal adjoining region, ASXM1 and ASXM2 domains in the middle region and a PHD domain in the C-terminal region (Figure 1C).

The ASXN domain, which we first discovered in 2003 based on the homology between human ASXL1 and ASXL2 (Katoh and Katoh, 2003), is conserved among vertebrate ASXL homologues but not *Drosophila* Asx. The ASXN domain is structurally similar to the Forkhead-box (FOX) domain, which is also known as the winged helix-turn-helix domain, of FOXA3, FOXK1, FOXO1 and FOXO4 (Sanchez-Pulido *et al*, 2012). The FOX domain is the DNA-binding module of the FOX family members, which are involved in transcriptional regulation and DNA repair during embryogenesis and carcinogenesis (Katoh *et al*, 2013). Sanchez-Pulido *et al* (2012) predicted that the ASXN domain functions as the DNA-binding module of the ASXL family members.

The region around the ASXH domain of ASXL1 directly binds to BAP1 (Scheuermann *et al*, 2010) and KDM1A (LSD1; Lee *et al*, 2010). BAP1 and UCH37/UCHL5 are de-ubiquitinating proteases that share a similar domain architecture consisting of UCH and ULD domains; the ASXH-similar domain of ADRM1/hRpn13 and the region around the ASXH-similar domain of NFRKB/INO80G directly bind to the ULD domain of UCH37/UCHL5 (Sanchez-Pulido *et al*, 2012). These facts indicate that the region around the ASXH domain is a protein–protein interaction

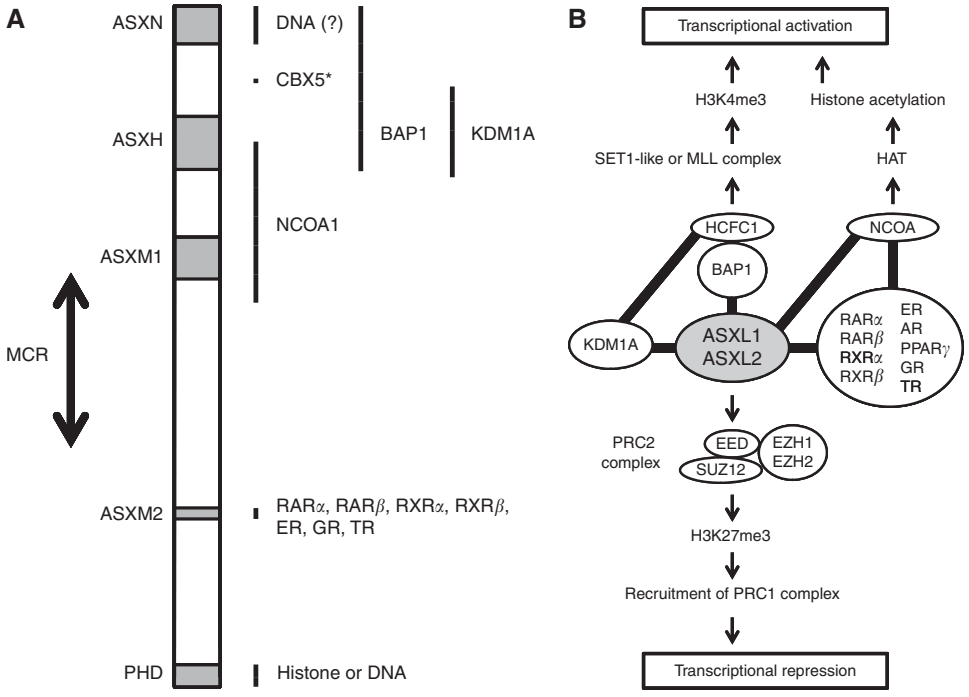

Figure 2. The ASXL network. (**A**) ASXL1-binding partners. The ASXN and PHD domains are binding modules for DNA or modified histones. The regions around the ASXH, ASXM1 and ASXM2 domains are binding modules for BAP1, KDM1A (LSD1), NCOA1 and NHRs, such as retinoic acid receptors and oestrogen receptor. CBX5 * indicates CBX5 binds to ASXL1, but not to ASXL2. Truncation mutations, clustered within the MCR region, give rise to aberrant ASXL proteins with ASXN and ASXH domains. (**B**) Involvement of the ASXL proteins in transcriptional regulation. ASXL proteins assemble BAP1, KDM1A, NCOA1 and NHRs to specific genomic loci with histone modification. ASXL proteins recruit BAP1 and NHR complexes for transcriptional activation, as well as PRC for transcriptional repression. The ASXL proteins activate or repress transcription of the target genes in a context-dependent manner.

module that associates with epigenetic regulators, such as BAP1 and KDM1A.

The ASXM1 and ASXM2 domains are also conserved among the ASXL family members (Figure 1C). The ASXM1 domain is located within the NCOA1 (SRC1)-binding region of ASXL1 (Cho *et al*, 2006). In contrast, the LVxxLL motif within the ASXM2 domain of ASXL1 is the binding motif for nuclear hormone receptors (NHRs), such as RARα, RARβ, RXRα, RXRβ, ER, AR, GR and TR (Cho *et al*, 2006). The ASXM1 and ASXM2 domains are protein–protein interaction modules that associate with NHRs and their cofactors.

The PHD domain, defined by a motif of four cysteine residues, one histidine residue and three cysteine residues (C4HC3), is a histone- or DNA-binding module of chromatin regulators and transcription factors (Sanchez and Zhou, 2011; Li and Li 2012; Liu *et al*, 2012). For example, the first PHD domain of BPTF, the third PHD domain of MLL1 and the PHD domains of ING1, PHF2, PHF8, PYGO1 and TAF3 all bind to tri- or dimethylated lysine 4 of histone H3 (H3K4me3/2); the first PHD domain of AIRE and the PHD domains of ATRX, DNMT3A and DNMT3L bind to unmodified lysine 4 of histone H3 (H3K4me0); the interface of the GATA-like finger and the PHD domain of ATRX binds to trimethylated lysine 9 of histone H3 (H3K9me3); the tandem PHD domains of KAT6A bind to acetylated lysine 14 of histone H3 (H3K14ac) and to unmodified arginine 2 of histone H3 (H3R2me0); the tandem PHD domains of DPF3 bind to H3K14ac and to H3K4me0/H3R2me0; the tandem PHD domains of BRPF2 bind to H3K4me0 and DNA (Sanchez and Zhou, 2011; Li and Li, 2012; Liu *et al*, 2012; references therein). Although the targets of the PHD domain of the ASXL family members remain to be identified, the PHD domain is predicted to be a histone- or DNA-binding module.

ASXL family proteins are divergent in non-conserved regions (Katoh and Katoh, 2003). For example, the CBX5 (HP1α)-binding PxVxL motif is located between the ASXN and ASXH domains of ASXL1 and ASXL3, but is absent from the corresponding region of ASXL2 (Lee *et al*, 2010). CBX5 interacts with H3K9me3 and ASXL1 using the Chromo (Chromo 1) and Chromoshadow (Chromo 2) domains, respectively. Functional divergence between ASXL1 and ASXL2 occurs as a result of CBX5 interaction with ASXL1 but not with ASXL2.

The ASXN and PHD domains are putative DNA- or histone-recognition modules, whereas the middle regions around the ASXH, ASXM1 and ASXM2 domains are protein–protein interaction modules assembling BAP1, KDM1A, NCOA1 and NHRs (Figure 2A). ASXL family members are epigenetic scaffolding proteins that recruit epigenetic regulators and transcription factors to specific genomic loci with histone modifications.

FUNCTION OF ASXL1

ASXL1 assembles various proteins, such as PRC2, NHRs and BAP1, based on protein–protein interactions. ASXL1–PRC2, ASXL1–NHR and ASXL1–BAP1 are ASXL1 sub-complexes involved in epigenetic and transcriptional regulation.

ASXL1 is co-immunoprecipitated with components of PRC2, including EZH2 and SUZ12, and re-expression of ASXL1 promotes H3K27me2/3 to partially inhibit the aberrant expression of the *HOXA*-cluster genes in *ASXL1*-null leukaemic cells (Abdel-Wahab *et al*, 2012). It remains unclear whether ASXL1 binds to a PRC2 component directly or indirectly; however, ASXL1 recruits PRC2 to specific genomic loci, where it adds the H3K27me3 repressive mark to further recruit PRC1 for transcriptional repression (Figure 2B).

ASXL1 directly binds to NHRs, such as RARs, RXRs, ER and AR, as well as to the NHR-coactivator NCOA1 (Cho *et al*, 2006; Grasso *et al*, 2012). On the basis of the NCOA1-mediated recruitment of histone acetyltransferase to the ASXL1–NHR complex, ASXL1 promotes NHR-dependent transcriptional activation (Figure 2B).

ASXL1 as well as ASXL2, BRCA1, FOXK1, FOXK2, HCFC1 and YY1 are binding partners of BAP1 (Jensen *et al*, 1998; Sowa *et al*, 2009; Scheuermann *et al*, 2010; Yu *et al*, 2010; Dey *et al*, 2012). ASXL1, ASXL2, BRCA1 and YY1 bind to the region around the ULD domain of BAP1, whereas HCFC1 binds to the HBM motif of BAP1. BAP1, the human orthologue of the *Drosophila* Calypso, is a nuclear de-ubiquitinating enzyme. Polycomb repressor complex 1 is involved in the mono-ubiquitination of histone H2A, whereas the human ASXL1–BAP1 complex and the *Drosophila* Asx-Calypso complex are involved in H2A de-ubiquitination (Scheuermann *et al*, 2010). The BAP1–HCFC1 complex is recruited to specific genomic loci based on interactions with BAP1-binding partners; HCFC1 then recruits SET1-like or MLL complexes for transcriptional activation via H3K4me3 (Figure 2B).

Asxl1 homozygous mutant (knock-out) mice were generated through integration of a neomycin-resistant cassette into exon 5 of the *Asxl1* gene using homologous recombination (Fisher *et al*, 2010a, b). The ASXH, ASXM1, ASXM2 and PHD domains are deleted from the Asxl1 protein of these mice due to insertion of the neomycin-resistant cassette at codon 90 of mouse Asxl1. The *Asxl1* knock-out mice are characterised by partial neonatal death. Newborn *Asxl1* knock-out mice show posterior transformation of cervical (C7) and thoracic (T13) vertebrae, as well as anterior transformation of cervical (C1 and C2) vertebrae, which correspond to the mammalian PcG and trxG phenotypes, respectively, (Fisher *et al*, 2010a). Adult *Asxl1* knock-out mice exhibit body-weight loss, splenomegaly and mild haematological phenotypes, such as altered differentiation frequencies of lymphoid and myeloid progenitors (Fisher *et al*, 2010b).

Taken together, these facts clearly indicate that ASXL1 is involved in both transcriptional activation and transcriptional repression in a context-dependent manner (Figure 2B).

FUNCTION OF ASXL2

ASXL2 promotes adipocytic differentiation of 3T3-L1 pre-adipocytes through enhancement of ligand-induced PPARγ activity, whereas ASXL1 suppresses adipogenesis through inhibition of PPARγ activity (Park *et al*, 2011). *Fabp4* is a representative target of PPARγ. ASXL2 upregulates *Fabp4* transcription based on the MLL1-associated active chromatin mark H3K4me3. In contrast, ASXL1 downregulates *Fabp4* transcription based on the CBX5-associated repressive chromatin mark H3K9me3. The CBX5-binding motif is absent in ASXL2 but present in ASXL1. As deletion of the CBX5-binding motif from ASXL1 results in the promotion of adipogenesis such as that associated with ASXL2, functional divergence of ASXL2 and ASXL1 is in part due to the lack of a CBX5-binding motif in ASXL2 (Park *et al*, 2011).

Asxl2 homozygous mutant (knock-out) mice were generated from an embryonic stem (ES) cell line, with the β-geo cassette integration into intron 1 of the *Asxl2* gene (Baskind *et al*, 2009). The β-geo reporter protein is expressed instead of wild-type Asxl2 because of aberrant splicing from exon 1 of the *Asxl2* gene, encoding the N-terminal 19 amino acids, to the gene-trap cassette. As wild-type *Asxl2* splicing is not completely blocked by the gene-trap cassette, the wild-type *Asxl2* transcript is minimally expressed in the *Asxl2* knock-out mice at a level that is 3% of control mice. The *Asxl2* knock-out mice are characterised by partial embryonic lethality and premature death owing to a defect in or retardation of growth (Baskind *et al*, 2009). The skeletal abnormalities in the sacral and thoracic vertebrae of the *Asxl2* knock-out mice are due

to posterior transformation (PcG phenotype) and anterior transformation (trxG phenotype), respectively. The bone mineral density is significantly decreased in the *Asxl2* knock-out mice, most likely due to low turnover osteopenia (Farber *et al*, 2011). In addition, dilated cardiomyopathy accompanied by a disarray of the cardiac myocytes and interstitial fibrosis is observed in the *Asxl2* knock-out mice, owing to epigenetic aberrations associated with the decreased level of H3K27me3 (Baskind *et al*, 2009).

These facts indicate that Asxl2 is also involved in transcriptional activation and repression in a context-dependent manner (Figure 2B).

HUMAN GENETICS OF ASXL1 AND ASXL3

Germ-line mutations of human *ASXL1* occur in Bohring-Opitz syndrome, which is characterised by mental retardation, cranio-skeletal features and feeding problems (Hoischen *et al*, 2011). Germ-line mutations of human *ASXL3* occur in a novel syndrome also characterised by mental retardation, cranio-skeletal features and feeding problems, which are partially similar to those observed in Bohring-Opitz syndrome and Cornelia de Lange syndrome (Bainbridge *et al*, 2013).

ASXL1 germ-line mutations are *de novo* nonsense or frame-shift mutations, resulting in ASXL1 truncation with loss of the NHR-binding ASXM2 domain and the C-terminal PHD domain. *ASXL3* germ-line mutations are *de novo* nonsense or frame-shift mutations, resulting in ASXL3 truncation with loss of the ASXM1, ASXM2 and PHD domains. It is noteworthy that the ASXN and ASXH domains are retained in the truncated ASXL1 and ASXL3 mutants involved in Bohring-Opitz and similar syndromes, respectively. Epigenetic aberrations and cancer predispositions related to germ-line mutations of *ASXL1* and *ASXL3* remain to be elucidated.

CANCER GENOMICS

Nonsense point mutations or frame-shift mutations of *ASXL1* occur in haematological malignancies, such as myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), MDS/MPN, acute myeloid leukaemia (AML) and chronic lymphocytic leukaemia (CLL; Carbuccia *et al*, 2009; Gelsi-Boyer *et al*, 2009; Quesada *et al*, 2011). Myelodysplastic syndrome includes refractory cytopenia with unilineage dysplasia, refractory anaemia with ring sideroblasts, refractory cytopenia with multilineage dysplasia, refractory anaemia with excess blasts, 5q-deletion syndrome and other conditions; MPN includes *BCR-ABL1*-positive chronic myelogenous leukaemia (CML), polycythaemia vera, primary myelofibrosis, essential thrombocythaemia and other conditions; MDS/MPN includes chronic myelomonocytic leukaemia (CMML), *BCR-ABL1*-negative CML and other conditions (Vardiman *et al*, 2009). Whole-exome sequencing analyses revealed that *ASXL1* is mutated in 2.9% of CLL cases (Quesada *et al*, 2011), whereas meta-analyses revealed that *ASXL1* is mutated in 45.3% of CMML cases, 34.5% of MPN cases, 30% of secondary AML cases, 16.2% of MDS cases and 6.5% of *de novo* AML cases (Gelsi-Boyer *et al*, 2012).

Asxl1 silencing cooperates with oncogenic *NRasG12D* to induce hepatosplenomegaly and progressive anaemia in mouse model experiments, emphasising the role of Asxl1 in myeloid malignancies (Abdel-Wahab *et al*, 2012). However, myeloid malignancies do not occur in *Asxl1* knock-out mice (Fisher *et al*, 2010b). ASXL1 and ASXL2 orthologues share a common domain architecture, and mouse *Asxl1* and *Asxl2* are co-expressed in ES cells, in all stages of embryos and in various types of adult tissues, suggesting that the suppression of myeloid malignancies in the *Asxl1* knock-out mice might stem from a functional redundancy between *Asxl1* and *Asxl2*. In addition, the ASXH domain is deleted from the mouse *Asxl1* knock-out allele, whereas this domain is retained in the human ASXL1 truncation mutants. Human ASXL1 mutants with ASXN and ASXH domains retain the ability to assemble BAP1 and its binding partners (Figure 2), thereby resulting in aberrant epigenetic regulation. The upregulation of *LRP6, CYP1B1, MAGI3* and *MPP6*, and the downregulation of *KISS1R* and *MLLT10* occur in AML with mutant ASXL1, compared with AML with wild-type ASXL1 (Metzeler *et al*, 2011). As ASXL1 truncations in human myeloid malignancies are misregulating mutations that alter the transcriptional landscape, *ASXL1* mutations are associated with a poor prognosis in patients with MDS, MPN and AML (Gelsi-Boyer *et al*, 2012).

ASXL1 is also frequently mutated in colorectal cancer (CRC) cell lines with microsatellite instability (MSI; Williams *et al*, 2010). The G8 mononucleotide repeat at nucleotide position c.1927 to c.1934 of the *ASXL1* gene is susceptible to the deletion or insertion of a G nucleotide as a result of MSI. The deletion of c.1934G (c.1934delG), which causes a frame-shift (Gly645ValfsX58), occurs in 5 of 11 CRC cell lines with MSI, whereas the duplication of c.1934G (c.1934dupG), which causes a frame-shift (Gly646TrpfsX12), occurs in 1 of 11 CRC cell lines with MSI.

Truncation mutations of the *ASXL* family genes occur relatively infrequently in other types of human cancers (Table 1). *ASXL1* and *ASXL2* are mutated in 2.0% and 6.0% of castration-resistant prostate cancers, respectively, (Grasso *et al*, 2012). *ASXL1* and *ASXL2* are each mutated in 1.0% of breast cancers (Stephens *et al*, 2012). *ASXL1* is mutated in 10% and 1.4% of liver cancers and head and neck squamous cell carcinomas, respectively, (Li *et al*, 2011; Stransky *et al*, 2011). *ASXL2* is mutated in 4.2% of pancreatic cancers (Jones *et al*, 2008) and *ASXL3* is mutated in 4.0% of melanomas (Berger *et al*, 2012).

EPC1-ASXL2 gene fusion occurs in adult T-cell leukaemia/lymphoma because of a der(10)t(2;10)(p23;p11.2) translocation (Nakahata *et al*, 2009). As the putative DNA-binding domain of ASXL2 is substituted by an SRF/RING1A-binding region in the EPC1-ASXL2 fusion protein, the *EPC1-ASXL2* gene fusion disrupts the function of wild-type ASXL2.

Gene amplification accompanied by overexpression of the amplified gene product is one of the mechanisms for activation of a proto-oncogene. *ASXL1* is amplified and overexpressed in 5.1% of cervical cancers (Scotto *et al*, 2008).

CONCLUSION

ASXL1, *ASXL2* and *ASXL3* are human homologues of *Drosophila Asx*. The ASXL proteins directly interact with BAP1, KDM1A, NHRs and NCOA1 to target specific genomic loci using histone modifications. ASXL1 is involved in transcriptional repression based on the recruitment of PRC2, and also in transcriptional regulation based on the recruitment of NHR and/or BAP1 complexes. Bifunctional effects on transcriptional regulation are evolutionarily conserved between mammalian ASXL family members and *Drosophila* Asx. Gene amplification, chromosomal translocation and truncation mutations of *ASXL* family members occur in various types of human cancers (Table 1).

Perspectives. How are the ASXL family members recruited to specific genomic loci? ASXLs are indirectly recruited to NHR-binding regions through their interactions with NHRs and are potentially recruited to specific genomic regions with histone modifications using their ASXN and PHD domains (Figure 2A). The ASXN domain is conserved among vertebrate ASXL family members, but that conservation does not extend to *Drosophila* Asx (Katoh and Katoh, 2003; Katoh and Katoh, 2004), which might

lead to a functional divergence of the ASXL homologues between vertebrates and *Drosophila* owing to an altered recognition of the target loci. The ASXL-binding genomic loci in various types of human cells should be systematically investigated using a chromatin-immunoprecipitation assay combined with next-generation sequencing analysis.

ASXL1 is amplified and overexpressed in cervical cancer. Overexpressed ASXL1 could recruit the BAP1–HCFC1 complex for the transcriptional activation of proto-oncogenes or PRC2 for the transcriptional repression of tumour suppressor genes. Functional reports on *ASXL1* amplification in cervical cancer or other types of squamous cell carcinomas are necessary to further understand the oncogenic potential of ASXL1 overexpression.

The *ASXL1* mutation rate is 3.2% in cytogenetically normal AML (CN-AML) patients who are younger than 60 years of age, but 16.2% in CN-AML patients who are 60 years of age or older (Metzeler *et al*, 2011), indicating that *ASXL1* mutations are detected preferentially in older patients. As the expression of *Asxl1* mRNA is significantly decreased in the brains of 18-month-old mice compared with that in the brains of 1-month-old mice (Jee *et al*, 2007), the expression of wild-type *ASXL1* mRNA might also be decreased in the malignant myeloid cells of older patients. Alternatively, microdeletion might preferentially occur in the wild-type *ASXL1* allele of older CN-AML patients as a result of the longer period that has elapsed following the acquisition of the *ASXL1* truncation mutation. The promoter methylation status and copy-number aberrations of the *ASXL1* gene need to be investigated because epigenetic silencing or deletion of the wild-type allele might be involved in the preferential detection of *ASXL1* mutations in older patients.

ASXL1 truncation mutations occur relatively frequently in CRC cell lines with MSI and in clinical samples of myeloid malignancies (Table 1). The coding G8 microsatellite (c.1927 to c.1934) of the *ASXL1* gene is susceptible to insertion or deletion of a G nucleotide (c.1934dupG or c.1934delG) in CRC with MSI (Williams *et al*, 2010), whereas the c.1934dupG mutation is the most frequent *ASXL1* mutation in myeloid malignancies (Gelsi-Boyer *et al*, 2012). As the c.1934dupG *ASXL1* mutation was identified as one of transcripts resistant to nonsense-mediated decay in CRC with MSI, the ASXL1 mutant protein derived from the c.1934dupG allele is predicted to be expressed as a C-terminally truncated protein. The contribution of MSI to the *ASXL1* truncation mutation in myeloid malignancies should be further investigated.

Somatic mutations of *BAP1* also occur in various types of human malignancies, and loss of BAP1 causes myeloid transformation (Dey *et al*, 2012). The ASXL family members, by interacting with BAP1, NHR and PRC complexes, are predicted to be tumour suppressive or oncogenic in a context-dependent manner. Integrative analyses of various types of human cancers are necessary to determine the *ASXL*-mutation landscape and to understand the consequent network aberrations as a platform for ASXL-targeted cancer therapeutics.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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