X-CHROMOSOME GENETICS AND HUMAN CANCER

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In mammals, the X chromosome is unique within the chromosome set. In contrast to the other chromosomes — for which two active copies are present — both male and female cells carry only one active X chromosome. This is because males have only one X chromosome and in females only one copy is active, a situation that leads to specific characteristics for genes located on this chromosome. How are the outcomes of genetic events involved in cancer — namely activation of oncogenes and inactivation of tumour suppressors — expected to be different when these genes are carried on the X chromosome rather than on autosomes?

UNISOMY
The state of an individual or cell carrying only one member of a pair of homologous chromosomes.

MOSAICISM
The occurrence in an individual of two or more cell populations of different chromosomal constitutions derived from a single zygote.

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The human X chromosome carries approximately 1,500 genes, some of which — like autosomal genes — represent potential targets for the common genetic alterations that are observed in human cancer cells. X-chromosome genes that are known to be associated with specific cancers are listed in TABLE 1. As for autosomal genes, genetic alterations involving the X chromosome that can lead to cancer include gains and losses of chromosomes, genomic rearrangements and mutations, which can lead to activation of oncogenes or loss of function of tumour suppressors.

However, when studying cancers that involve genes on the X chromosome, it is important to keep in mind the unique status of this chromosome within the chromosome set. In mammals, male cells have only one X chromosome (a situation known as genetic UNISOMY), and in XX female cells one of the X chromosomes undergoes inactivation, which silences most of the genes encoded on this chromosome (functional unisomy). In this review, we integrate the unique features of X-chromosome genetics with the characteristics of cancer biology.

X-chromosome inactivation

In mammalian cells, gene dosage — the number of copies of a particular gene in the genome — must be finely tuned. Aneuploidy — the condition of having fewer or more than the normal diploid number of chromosomes — perturbs this dosage and is usually deleterious, particularly when it affects the number of autosomes. By contrast, although lack of an X chromosome in female cells has severe effects, females with supernumerary X chromosomes are often normal or nearly normal because of X-chromosome inactivation — a mechanism whereby XX female mammals equalize the dosage of X-linked genes with that in XY males. The hypothesis of X-chromosome inactivation was first proposed in 1961 by Mary Lyon in a study in mice, following the earlier observation by Barr and Bertram of a compact structure at the nuclear periphery in neurons of female cats[1]. The finding that this structure — known as the Barr body — contains one of the two X chromosomes of the female mammalian nucleus led Lyon to postulate that equalization of X-linked gene dosage between male and female mice is achieved by the transcriptional silencing of one X chromosome[2]. This finding was further extended to females of other mammals, including humans.

X-chromosome inactivation (FIG. 1), which is initiated early in embryonic stem-cell differentiation, is characterized by three features: first, a counting process ensures that all but one of the X chromosomes undergo inactivation; second, the choice of the inactivated X chromosome is random, so that the X chromosome inherited from each parent is silenced in 50% of cells; third, this choice is 'memorized', so that the process is heritable through subsequent rounds of cell division[3]. As a result, in female mammalian somatic tissues, one X chromosome is active and the other is transcriptionally silenced. These tissues are therefore MOSAIC with respect to whether the maternal or paternal X chromosome is active[3].

Summary

- Although female mammals carry two copies of the X chromosome, both male and female mammalian cells carry a single active X chromosome, as in females one copy of the X chromosome is inactivated.
- Both of the main types of genetic alterations that lead to cancer — tumour-suppressor inactivation and oncogene activation — act dominantly when they affect the single active copy of an X-linked gene. The same alterations remain silent when they affect the inactivated X chromosome in female cells.
- Increased dosage of X-linked genes is thought to represent a key event in oncogenesis. Two principal mechanisms that achieve such change in gene dosage are commonly observed in tumours: gain of whole copies or regions of the active X chromosome and loss or skewing of the inactivation mechanism.
- As for autosomal genes, the expression of X-linked genes can be altered by changes in methylation, in addition to classic genetic mutations. Increases and decreases in methylation of X-chromosome genes have been implicated in certain cancers.
- Some genes that are located on the inactive X chromosome escape inactivation in normal cells and several of these are implicated in human cancer.
- Translocations involving regions of the X chromosome have unique outcomes in relation to ability to cause cancer. Events involving relocation of regions of the inactive X chromosome to an autosome can result in the reactivation of previously silent X-linked genes, with potential oncogenic effects. Conversely, loss of expression of an autosomal tumour suppressor can result from translocation to the inactive X chromosome.
- Defects in the X-chromosome inactivation process can lead to cancer. The *BRCA1* tumour suppressor is thought to have a key role in X-chromosome inactivation, and it has been proposed that loss of this function contributes to the development of cancer when normal expression of this gene is lost.

The silencing of the inactivated X chromosome results from sequential epigenetic modifications involving various key effectors[3] (FIG. 1). A detailed description of this process is beyond the scope of this review, and more detail is given in REF. 3. Briefly, the process is initiated by the transcription on the designated X chromosome — that is, the X chromosome that will ultimately be inactivated — of *XIST* (X-inactive-specific transcript) RNA, a 17-kb spliced and polyadenylated RNA with no coding capacity. *XIST* RNA remains confined to the nucleus, where it spreads *in cis* on the chromosome from which it is expressed (see FIG. 1 and below). This coating provides the template for a series of histone modifications, including histone-H3 methylation, histone-H4 deacetylation and histone-macroH2A accumulation. This sequential process is regulated in *cis* by the X-chromosome inactivation centre (*XIC*), which controls X-chromosome counting, the choice of which X chromosome is inactivated and the initiation of silencing. As a consequence of this process, approximately 85% of the X chromosome acquires the features of HETEROCHROMATIN, which is characterized by transcriptional silencing. For the 15% of genes that escape inactivation, both alleles are expressed. These genes are not randomly distributed, as 80% of them lie on the short arm (the p arm) of the X chromosome (BOX 1).

HETEROCHROMATIN
Highly condensed region of the interphase nucleus consisting of nucleic acid and associated histone proteins packed into nucleosomes. Heterochromatin is transcriptionally inactive and becomes especially abundant in the nuclei of terminally differentiated cells, in which most formerly active genes are repressed.

LOSS OF HETEROZYGOSITY
Refers to a mutation or other genetic event that results in the loss of one allele.

Impact of X inactivation on cancer genetics

In addressing how alterations of X-linked genes might contribute to cancer, the genetic hallmarks of cancer should be emphasized. Like other diseases, cancer can be initiated by a single genetic event occurring either in somatic cells or in germ cells, leading to sporadic cancers or hereditary cancers, respectively. However, unlike most other diseases, cancer usually begins as a clonal growth, reflecting a selective advantage brought about by genetic alterations that occur in a single cell. Because of X-chromosome unisomy (genetic unisomy in males and functional unisomy in females), the common genetic events that cause cancer — oncogene activation and tumour-suppressor inactivation — are expected to produce different results when they affect genes that are carried on the X chromosome to those produced when they affect autosomal genes (FIG. 2). Gain-of-function mutations leading to oncogene activation — which acts dominantly when it affects autosomal genes — might remain silent if they occur on the inactivated allele of an X-linked gene. By contrast, tumour-suppressor gene inactivation by loss of a single allele (loss of function), which is recessive when it affects autosomal genes, might become dominant when the other allele is functionally silenced as a result of X-chromosome inactivation. X-chromosome inactivation therefore represents an operational LOSS OF HETEROZYGOSITY (LOH).

The same effects of X-chromosome inactivation apply to germline mutations. X-chromosome inactivation occurs early during embryonic differentiation and randomly silences either the maternal or paternal X chromosome, resulting in the mosaicism of female adult tissues with respect to which copy of the chromosome is active. If the random nature of the X-chromosome inactivation is preserved, adult tissues from carriers of a germline mutation in an X-chromosome-encoded gene will therefore be mosaic with respect to the mutation-associated phenotype. If the inactivation is skewed (non-random; see below) — favouring, for instance, inactivation of the mutated X chromosome — the mutation-associated phenotype will be expressed in a minority of tissue compartments. In the case of cancer, only the compartments carrying an activated oncogene or an inactivated tumour suppressor on the active X chromosome will be prone to cancer.

Combinations of gains and losses of different functions confer selective growth advantages to cancer cells. Gains of function can be achieved through several mechanisms. These include activating mutations; increases in gene dosage resulting from copy-number amplification; gains of whole chromosomes or chromosomal regions; and increases in expression of certain genes resulting from loss of regulation. This dysregulation can result from mutations in promoters or translocations to regions downstream of promoters of other genes. In addition to these processes, other mechanisms can operate specifically on X-linked genes. These include reactivation of regions on the inactive X chromosome following translocation onto an autosome, deficiencies in the inactivation process and duplication of an X chromosome. Similarly, loss-of-function effects that lead to cancer can be achieved through several pathways for X-chromosome genes. These include inactivating mutations, deletions (loss of heterozygosity) and epigenetic modifications. Specifically for X-chromosome genes, it has been demonstrated that a translocation of a segment of the inactive X chromosome to an autosome can potentially silence an autosomal region in *cis*. This position effect, which is due to heterochromatinization, might not require *XIC*, and there is conflicting data as to whether the ectopic introduction of *XIST* leads to *de novo* inactivation in somatic cells.

An extensive body of literature relating to the X chromosome and cancer is available. Some of this goes back to the 1950s and reports pathological observations, with attempts to link the presence or the absence of an inactive X chromosome to prognosis in human cancers[4,5]. More recent reports describe

Table 1 | **X-chromosome genes involved in human cancer**

Locus	Gene	Function of protein product	Involvement in cancer
Xp11.2	*ELK1*	Member of ETS family of transcription factors; direct target of the MAPK pathway	Upregulated in testicular germ-cell tumours and urothelial cancers
Xp11.2	*BMP15*	Regulates granulosa-cell proliferation and differentiation	Possible involvement in ovarian tumours
Xp11.23	*UBE1**	Ubiquitylation	Downregulated in acute promyelocytic leukaemia
Xp11.23	*PIM2*	Serine/threonine kinase	Upregulated in mantle-cell lymphomas and multiple myelomas
Xp11.3–11.23	*TIMP1*	Metalloproteinase inhibitor	Upregulated in anaplastic large-cell lymphomas, liposarcomas, bladder tumours, desmoid tumours, squamous-cell lung cancers, breast cancers and hepatocarcinomas
Xp11.4–11.2	*ARAF1*	Serine/threonine kinase	Upregulated in lung adenocarcinomas and testicular germ-cell tumours
Xp22.2	*BMX*	Non-receptor tyrosine kinase	Upregulated in breast cancers, prostate adenocarcinomas and myeloid leukaemias
Xp22.2–22.13	*GRPR**	G-protein-coupled receptor	Upregulated in gastrointestinal carcinoid tumours, colon adenocarcinomas, head and neck squamous-cell carcinomas, ovarian cancers, prostate adenocarcinomas and non-small-cell lung cancers
Xp22.22	*RBBP7**	Regulation of cell proliferation	Involvement in acute promyelocytic leukaemias (mechanism unknown)
Xp22.31	*PIR**	Transcription cofactor	Upregulated in salivary-gland adenoid cystic carcinomas
Xp22.32	*CD99*	Cell-adhesion molecule	Upregulated in desmoplastic small-cell tumours, synovial sarcomas, primitive neuroectodermal tumours and hepatocarcinomas
Xq11.2–12	*MSN*	Structural component of cytoskeleton	Upregulated in skin carcinomas
Xq11.2–12	*AR*	Androgen receptor	Upregulated in prostate adenocarcinomas
Xq12	*EFNB1*	Involved in cell adhesion	Upregulated in gastric adenocarcinomas and ovarian adenocarcinomas
Xq24–27	*BZX*	Unknown	Possible involvement in small-cell lung cancers
Xq25	*HDGF*	Involved in heparin-binding associated with cell proliferation	Upregulated in hepatocarcinomas
Xq26.1	*GPC3*	Involved in cell growth and/or maintenance	Upregulated in embryonal tumours, rhabdomyosarcomas, hepatoblastomas, Wilm's tumours, Ewing's sarcomas, hepatocarcinomas and gastric adenocarcinomas
Xq26.2	*MST4*	Serine/threonine kinase	Upregulated in prostate adenocarcinomas
Xq26.3	*FGF13*	Involved in cell–cell signalling	Upregulated in fibrosarcomas and melanomas
Xq27	*TGCT1*	Unknown	Involved in testicular germ-cell tumours (mechanism unknown)
Xq27–28	*HPCX*	Unknown	Involved in prostate adenocarcinomas (mechanism unknown)
Xq28	*MECP2*	Transcription corepressor	Upregulated in colorectal adenocarcinomas
Xq28	*RPL10*	Tumour suppressor	Downregulated in prostate adenocarcinomas

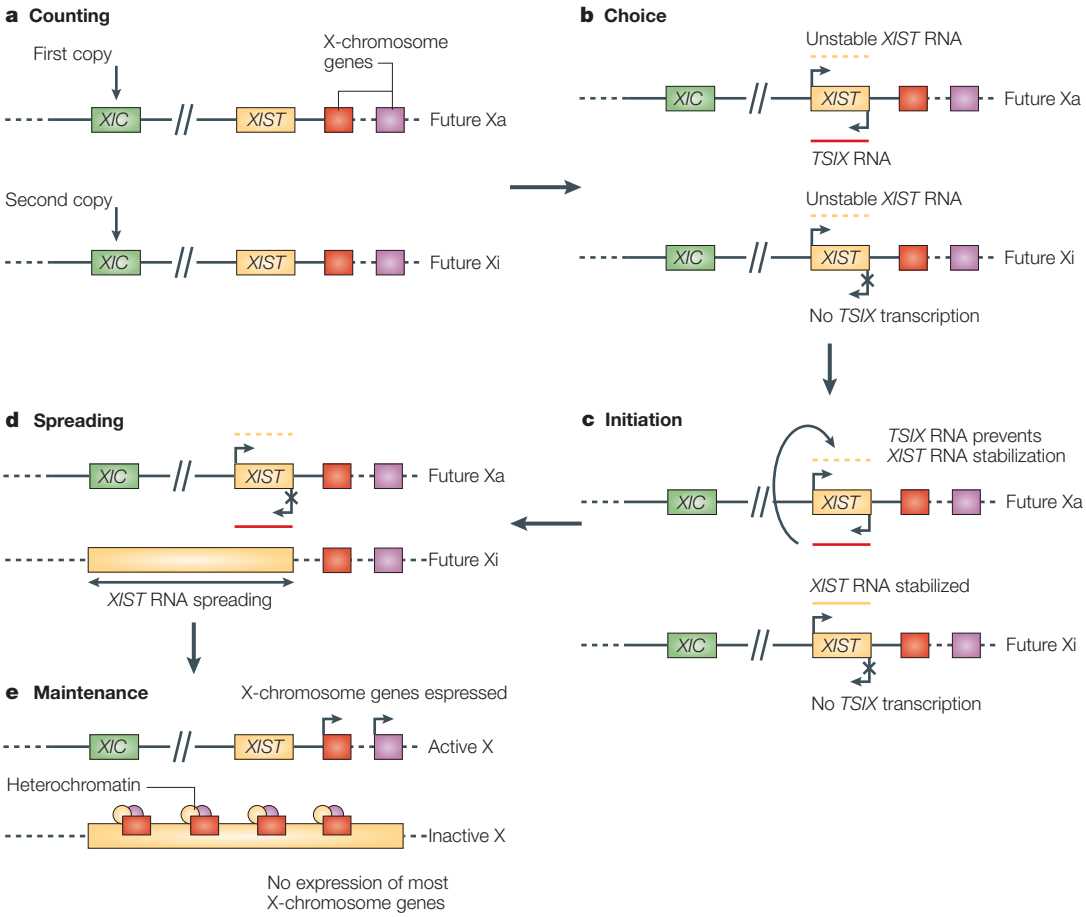
*Genes that escape X-chromosome inactivation. *ARAF1*, *v-raf* murine sarcoma viral oncogene homologue 1; *BMP15*, bone morphogenetic protein 15; *BZX*, Bazex syndrome; *EFNB1*, ephrin B1; *FGF13*, fibroblast growth factor 13; *GPC3*, glypican 3; *GRPR*, gastrin-releasing peptide receptor; *HDGF*, hepatoma-derived growth factor; *HPCX*, hereditary postate cancer predisposition gene; MAPK, mitogen-activated protein kinase; *MECP2*, methyl CpG-binding protein 2; *MSN*, moesin; *MST4*, Mst3 and SOK1-related kinase; *PIR*, pirin; *RBBP7*, retinoblastoma-binding protein 7; *RPL10*, ribosomal protein L10; *TGCT1*, testicular germ-cell tumour susceptibility 1; *TIMP1*, tissue inhibitor of metalloproteinase 1; *UBE1*, ubiquitin-activating enzyme E1.

attempts to identify putative oncogenes, tumour-suppressor genes and tumour-antigen genes on the X chromosome (TABLE 1). However, these studies rarely take into account the X-chromosome inactivation process in females or X-chromosome unisomy in males. The following sections attempt to integrate what is known about X-chromosome biology with studies of various cancers involving X-linked genes.

Increased dosage of X-chromosome genes

In contrast to normal cells, which are essentially diploid, aneuploidy is a frequent hallmark of tumour cells, indicating that changes in gene copy number might represent a key pathway to oncogenesis. Gene copy number can also be altered by many other mechanisms, such as gains in chromosomal arms or amplifications of regions carrying specific genes. In all these events, the acquisition of oncogenic properties by normal gene products relies on the effects of increased gene dosage. In this section, we will describe all of these types of gain involving genes of the X chromosome and will discuss the results of these gains when they occur both in the germline and in somatic cells.

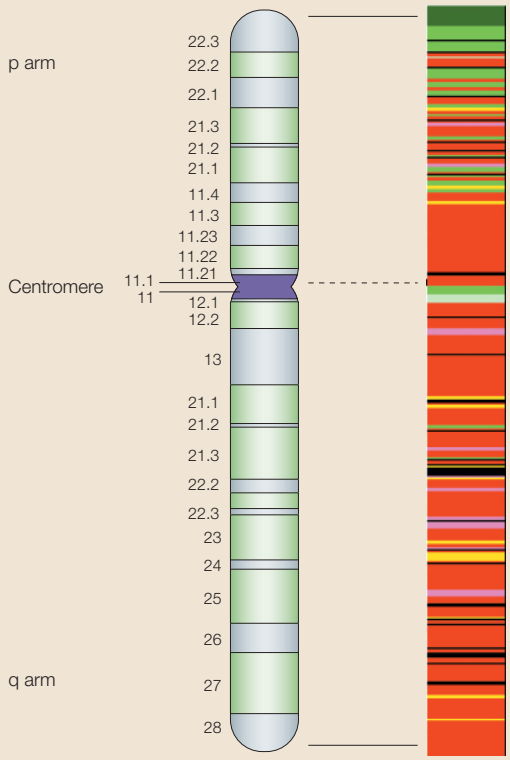
Germline increases in X-chromosome gene dosage. Gains of autosomes that affect germline cells or cells of the early zygote (and are therefore represented in all cell compartments of the body) are rare events in humans and are usually associated with severe developmental abnormalities. This indicates that a strong selective pressure is exerted to maintain the correct number of autosomes during normal development. Interestingly, the pressure on X-chromosome ploidy is much less stringent. Indeed, syndromes that are characterized by the presence of multiple X chromosomes are common (XXX females and XXY KLINEFELTER'S SYNDROME males each



KLINEFELTER'S SYNDROME
A syndrome affecting males, characterized by small testes, infertility and the development of breasts. Patients tend to be tall with long legs. The syndrome is typically associated with an XXY chromosome complement, although variants include XXYY, XXXY, XXXXY and several mosaic patterns.

Figure 1 | **The process of X-chromosome inactivation.** Stable transcriptional silencing of the inactivated X chromosome (Xi) is achieved through a multistep process. **a** | First, a locus on the X chromosome known as the X-chromosome inactivation centre (*XIC*) is 'counted', as at least two copies of *XIC* must be present per diploid genome in order for inactivation to occur. The counting process ensures that one X chromosome remains active in diploid cells. **b** | Both X chromosomes transcribe *XIST* RNA, which is required for X-chromosome inactivation. The choice of which X chromosome is inactivated is determined by asymmetric expression of *TSIX* RNA from the opposite strand to *XIST* only on the future active X chromosome (Xa). *TSIX* transcription is required to restrict *XIST* activity on the future active X chromosome. Conversely, *TSIX* repression is required for *XIST* RNA accumulation on the future Xi chromosome. **c** | Initiation of X-chromosome inactivation is mediated by stabilization of *XIST* RNA on the future Xi, whereas the Xa-chromosome allele transcribes *XIST* RNA that it is not subsequently stabilized. **d** | Stable *XIST* RNA spreads in *cis* on the chromosome from which it is expressed. **e** | This coating of the DNA with *XIST* provides the template for a sequence of histone modifications that promote heterochromatin formation, eventually leading to transcriptional silencing and ensuring the maintenance of X-chromosome inactivation.

Box 1 | **Structure of the X chromosome and patterns of gene inactivation**



Structure

As for the autosomes, the X chromosome consists of a short arm (p) and a long arm (q) separated by the centromere. The structure of the human X chromosome, indicating its low-resolution banding pattern, is shown on the left in the figure. Regions of the X chromosome are referred to according to their location. For example, the gene that encodes the androgen receptor is located in the region containing bands 11.2 and 12 on the q arm of the X chromsome, a region referred to as Xq11.2–12.

Patterns of gene inactivation

Although one of the two X chromosomes in female cells is subject to X-chromosome inactivation, not all genes on this chromosome are transcriptionally silenced. The distribution of X-chromosome genes with regard to their activation status is shown in the right-hand part of the figure. Approximately 85% of the X chromosome acquires a status of heterochromatin, which is characterized by transcriptional silencing. For the 15% of genes that escape inactivation, the alleles on both of the X chromosomes are expressed. These genes are not randomly distributed, as 80% of them lie on the p arm, mainly in the distal region (that is, the region furthest from the centromere). Figure compiled from data in REF. 62**.**

represent 1/1000 female or male births, respectively) and are characterized by relatively mild phenotypes. This striking difference reflects the role of the X-chromosome inactivation process, which regulates gene dosage by silencing all but one X chromosome, therefore minimizing the effects of extra copies. However, some genes escape inactivation and their dosage is therefore increased in cells that are polyploid for the inactivated X chromosome (TABLE 1). Whether the increase in gene dosage for the genes that escape inactivation is associated with pathogenic situations will be discussed below.

The involvement of X-chromosome gains in oncogenesis is illustrated by male breast cancer. This condition is rare, accounting for less than 1% of all breast cancers. Cytogenetic analyses have shown consistent gains of X chromosomes in these tumours[6,7]. Furthermore, both XXY Klinefelter's syndrome and XX MALE SYNDROME are known risk factors for male breast cancer[8,9]. In the case of these germline aneuploidies, the counting process is expected to achieve the correct gene dosage by inactivating all but one X chromosome. However, in this setting, the origin of the supernumerary X chromosome — whether it is paternal or maternal — could be important. For example, in mice, the paternal X chromosome undergoes IMPRINTED X-chromosome inactivation before cellular differentiation, which is erased at a later stage of development, when inactivation becomes random[10,11]. Moreover, it has been observed that in XXY cells from patients with Klinefelter's syndrome, X-chromosome inactivation is often skewed (non-random), showing preferential inactivation of one of the X chromosomes (see below)[12]. Whether skewed X-chromosome inactivation can account for the wide range of phenotypic abnormalities that are observed in Klinefelter's syndrome and XX male syndrome — including breast cancer risk — remains to be elucidated. In females, the situation is different, as only a small number of cancer cases have been reported in either XXX or XXXX females. In addition, there is no evidence so far for either an increase in risk for any cancer, including breast cancer, or for hormonal imbalances in these patients[13].

Somatic increases in X-chromosome gene dosage. All kinds of X-chromosome gains — gains of whole chromosomes or chromosome arms, and gene amplifications — have been observed in many types of solid and haematopoietic tumours. For instance, an increase in X-chromosome number is a common feature of testicular germ-cell tumours (TGCTs)[14]. It is noteworthy that a candidate TGCT-susceptibility gene of unknown function, *TGCT1*, has been localized to Xq27 and seems to be associated with a risk of bilateral disease[15]. A high frequency of gains in one

XX MALE SYNDROME
A syndrome that occurs in males that is associated with the presence of two X chromosomes. The parts of the Y chromosome that are necessary for the male phenotype are thought to be located elsewhere in the genome as a result of translocation, at least in some cases.

IMPRINTING
Monoallelic gene expression or inactivation of either the maternal or paternal allele of a particular locus.

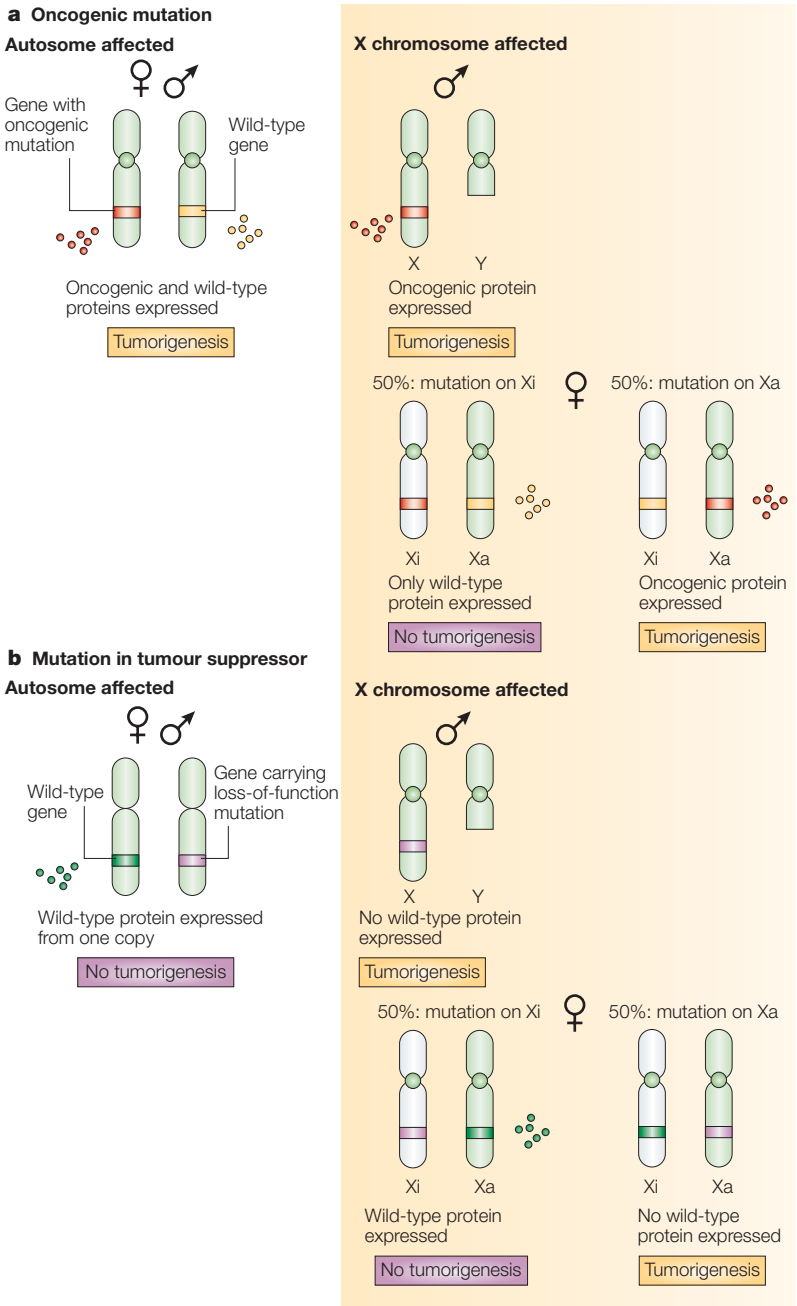


Figure 2 | **The impact of X-chromosome genetics on cancer.** Inactivating mutations in tumour-suppressor genes and activating mutations in oncogenes have different effects when they target the X chromosome rather than an autosomal gene. **a** | An oncogenic mutation of an autosomal gene will lead to tumorigenesis, as the oncogenic form of the protein is expressed. A different result is seen if such a mutation affects an X-chromosome gene. In males, this again leads to tumorigenesis, as the oncogenic form will always be expressed from the single copy of the X chromosome. In females, a mutation in an oncogene carried on the inactive X chromosome (Xi) will have no deleterious effect if this gene does not escape X-chromosome inactivation. However, if the mutation affects the active copy of the X chromosome (Xa), tumorigenesis will occur. **b** | Mutation of a single copy of an autosomal tumour-suppressor gene does not lead to tumorigenesis, as the functional copy on the sister chromosome compensates for this loss. Again, the effect is different when an X-linked tumour-suppressor gene is involved, as cells contain only one active copy of most X-chromosome genes. In males, for genes for which there is no Y-chromosome homologue, a single event will lead to complete loss of tumour-suppressor activity and tumorigenesis, as no second copy of the gene is present. In females, mutation of a tumour suppressor will have no effect if it targets the inactive X chromosome. However, for genes that do not escape inactivation on the inactive X chromosome, a single loss-of-function event that affects a tumour suppressor will lead to tumorigenesis if the active X chromosome is affected, as the only wild-type copy of the gene is inactivated.

arm of the X chromosome, as assessed by comparative genomic hybridization, has also been observed in hepatoblastomas. 43% of these tumours show gains in Xp and 60% show gains in Xq, underlining the possibility that X-linked genes are involved in the development of this type of neoplasm[16].

It has been suggested recently that the gain of an extra X chromosome might have an important role in the progression from chronic phase to blast crisis in chronic neutrophilic leukaemia[17]. Similarly, a cytogenetic study of 75 children with acute lymphoblastic leukaemia (ALL) revealed an acquired extra X chromosome in 29 (88%) of 33 children with a high hyperdiploid karyotype (> 50 chromosomes) and in 4 (33%) of 12 children with a low hyperdiploid karyotype (47–50 chromosomes)[18]. Altogether, 57.3% of newly diagnosed children displayed X-chromosomal aneuploidy. This indicates that X-chromosome aneuploidy is likely to be the most common chromosomal abnormality in childhood ALL, and might have an important role in its development.

The gain of an additional X chromosome has also been reported in prostate carcinomas. In this case, X-chromosome gains are associated with a selective growth advantage, which supports tumour progression[19]. It has been shown that increases in X-chromosome number or specific amplifications of the androgen-receptor (*AR*) gene located at Xq11.2–12 are associated with cases of cancer recurrence [20,21]. More specifically, 30% of patients with prostate cancer who are treated by anti-androgen therapy carry such an amplification of *AR*, which results in the facilitation of tumour growth in low androgen concentrations[22]. However it should be emphasized that no direct evidence for overexpression of X-derived transcripts has yet been provided for any of the cases described above in which somatic gains in X chromosomes are associated with cancer.

Gains in active versus inactive X chromosomes. Assuming that gains in X-chromosome number confer growth or survival advantages in tumour progression, the inactivation status of the supernumerary chromosomes is worth considering. In conditions where the silencing process operates efficiently, no selective advantage is expected for gains of an inactive X chromosome, and over-representation of active X chromosomes should therefore be the rule. Indeed, this is the case for colorectal and anal-canal tumours, in which somatic imbalances between sex chromosomes have been observed[23,24]. These imbalances include gain of an additional active X chromosome in both males and females. Similarly, breast cancer cells commonly display two identical active X chromosomes, a status resulting from duplication of the active X chromosome, which might or might not be associated with the simultaneous loss of the inactive chromosome[25]. In addition, gains of hypomethylated, presumably active, X chromosomes occur in nearly all intracranial germ-cell tumours[26]. Finally, the multiple X chromosomes that are present in TGCTs can be either active or inactive,

and both situations could be associated with an oncogenic effect. It has been shown that an excess of active X chromosomes in TGCTs correlates with increased expression of two X-linked oncogenes — *ARAF1* and *ELK1* — in some TGCT-derived cell lines, indicating a biological significance of an excess of active X chromosomes[27]. On the other hand, as some genes on the inactive X chromosome escape silencing, increases in the number of inactive X chromosomes could also be oncogenic. In support of this hypothesis, Looijenga *et al.* reported that X-chromosome inactivation — characterized by *XIST* expression and the typical DNA-methylation pattern of the inactive X chromosome — is operational in some TGCTs that have supernumerary X chromosomes[14,28,29]. However, a direct contribution of additional active X chromosomes to the development of TGCTs has not been demonstrated so far.

Tumour-suppressor genes on the X chromosome

LOH at a specific chromosomal region is a hallmark of the presence of a recessive tumour-suppressor gene. As discussed earlier, because mammalian cells contain only one active X chromosome, a single event that affects a tumour-suppressor gene should be sufficient to lead to loss of function. Strong evidence for the presence of a putative tumour-suppressor gene on the X chromosome was provided in the early 1990s by X-chromosome-transfer experiments. The introduction of an X chromosome into a nickel-transformed chinese-hamster cell line carrying a deletion on the q arm of one X chromosome resulted in the senescence of these otherwise immortal cells[30,31]. This prompted a search for inherited LOH involving X-linked loci in cancer cells.

In a population-based cohort study, Monroe *et al.* reported an increased risk of prostate cancer in men with affected brothers compared with those whose fathers were affected, raising the possibility of X-linked inheritance[32]. The X-linked inheritance of familial prostate cancers was confirmed by Xu *et al.* in a population-based study of 360 families with cases of prostate cancer[33], providing evidence for a human prostate-cancer-susceptibility locus on the X chromosome located at Xq27–28, which was subsequently termed the *HPCX* gene. This gene might also have a role in sporadic prostate cancer, in which there is a deletion at Xq27 (REF. 34). The functions of *HCPX* are not yet known. Interestingly, the *TGCT1* susceptibility gene for familial TGCTs has also been ascribed to Xq27 (REFS 15,35,36).

LOH affecting the X chromosome has also been implicated in several other cancers. In the case of female breast adenocarcinomas, Piao *et al.* reported a recurrent loss of an X-chromosome locus (at Xq25) in 52% of 72 infiltrating ductal carcinomas[37]. This X-chromosome LOH was correlated with larger tumour size, higher histological grade and axillary lymph-node metastasis, supporting a role for inactivation of a putative tumour-suppressor gene leading to tumour progression. Since 1992, there has also been increased interest in the role of X-chromosome imbalances in ovarian cancers, as LOH on the X chromosome is seen in ~40% of sporadic ovarian cancers[38–43]. Frequent losses are observed at the Xq25–26 region in these cancers, and are significantly associated with high nuclear grade[42].

The involvement of LOH on the X chromosome is not limited to sex-organ-specific cancers. X-chromosome LOH might also be associated with tumour aggressiveness and prognosis in renal-cell carcinoma[44]. Papillary renal-cell carcinomas are associated with LOH that occurs due to acquired chromosomal translocations involving the *TFE3* gene that is located on the X chromosome[45]. These translocations lead both to LOH for a region of the X chromosome and to the generation of oncogenic fusion proteins, which consist partly of the helix–loop–helix leucine-zipper region of TFE3. Type-2 papillary renal-cell carcinoma, which is associated with a high nuclear grade and aggressive disease, shows more frequent X-chromosome LOH when compared with less aggressive type-1 tumours. LOH involving the X chromosome has also been reported in all metastatic gastric carcinoid tumours derived from ENTEROCHROMAFFIN-LIKE CELLS, whereas localized carcinoids did not show X- chromosome LOH[46]. These results have been further extended to gastroenteropancreatic (GEP) endocrine tumours[47]. LOH was seen in 60% of infiltrative and metastatic GEP tumours, compared with 4.5% in benign neoplasms. Altogether, these results indicate that an X-linked tumour-suppressor gene is targeted for mutation in foregut endocrine neoplasms.

More work needs to be done to identify the specific genes targeted by LOH in the cancers mentioned above, which should confirm these genes as tumour suppressors. Such studies will also enable the status of the retained allele to be determined, in terms of whether it is wild-type or mutant. This will address the issue of the number of inactivation events that are required for tumorigenesis in these cancers — that is, whether a double hit is required, as for autosomal tumour suppressors, or a single hit, as predicted for tumour suppressors located on the active X chromosome.

Alterations in X-chromosome methylation

It is widely accepted that epigenetic modifications of chromatin have a key role in the regulation of gene expression, in both the activation and silencing of genes. The chromatin remodelling that results from epigenetic changes involves post-translational histone modifications — mainly acetylation, methylation and phosphorylation — and DNA methylation[48]. Any alteration in these pathways has a direct impact on changes in gene activity and, therefore, on cancer onset.

Regions of the genome containing methylated promoters correlate with regions that contain inactive genes, whereas regions of the genome containing unmethylated promoters contain active genes. Methylation-mediated gene silencing is thought to occur through the blockage of the transcriptional machinery following chromatin remodelling. Cancer cells are characterized by global hypomethylation of genomic DNA[49], which directly contributes to the activation of oncogenes or latent RETROTRANSPOSONS[50] and generalized chromosome instability. However,

ENTEROCHROMAFFIN-LIKE CELL
A distinctive type of neuroendocrine cell present in gastric mucosa underlying epithelia; most prevalent in the acid-secreting regions of the stomach.

RETROTRANSPOSONS
Transposable elements (transposons) that, similar to retroviruses, require reverse transcription for their replication. The DNA element is transcribed into RNA, reverse-transcribed into DNA and then inserted at a new site in the genome.

cancer cells can also acquire specific hypermethylated sites that could contribute to cancer progression either through genetic instability — leading to breakage and recombination — or through inactivation of a tumour-suppressor gene[49]. Although this mechanism of epigenetic control of gene expression is not specific to the X chromosome, because of X-chromosome unisomy, epigenetic changes on this chromosome might lead to different effects to similar changes on autosomes, similar to the situation for genetic loss- and gain-of-function mechanisms.

Studies of the glypican 3 gene (*GPC3*), which is located on the X chromosome at Xq26, illustrate the role of epigenetic modifications of X-linked genes in human cancer. GPC3 is a heparan-sulphate proteoglycan that can induce apoptosis and acts as an inhibitor of cell proliferation or as a negative regulator of insulin-like growth factor 2 signalling[51]. *GPC3* is mutated in patients with Simpson–Golabi–Behmel overgrowth syndrome, an X-linked hereditary disorder associated with skeletal and visceral-organ malformations[51] and increased risk of childhood tumours[52]. Normal expression of GPC3 is restricted to mesothelial, ovarian and mammary tissues, and GPC3 expression is downregulated in cancers arising from these tissues (such as malignant mesotheliomas[53] and ovarian adenocarcinomas[54]) and in breast cancers[55], indicating that it might have tumour-suppressor activity. In each of these types of tumour, no mutations have been found in the *GPC3* coding region, leading to the suggestion that downregulation could be accounted for by hypermethylation of the promoter[53]. Evidence for this comes from experiments in cell lines, which have shown that suppression of hypermethylation by treatment with the demethylating agent 5-aza-2′deoxycitidine restored GPC3 expression.

Alterations in methylation are also implicated in the deregulation of antigens expressed in testicular cancers, which are mostly encoded by genes located on the X chromosome. These proteins belong to the cancer/testis (CT) antigen family, and their expression is predominantly restricted to normal testis and is silenced in other normal tissues. As well as being overexpressed in testicular cancer, the expression of these proteins is also reactivated in various other types of cancer, especially melanomas. Twelve genes coding for melanoma-associated antigens (genes of the MAGEA group), which belong to the CT antigen family, are located at Xq28, and the *MAGEB1*, *MAGEB2*, *MAGEB3* and *MAGEB4* genes are located on Xq21.3. Altogether, 23 human and 12 mouse MAGE genes have been found to be overexpressed in various tumours, and have subsequently been characterized. Little is known about their function in normal cells, although their specific expression in the testis indicates that this gene family is involved in germ-cell development. The CT-antigen family is constantly increasing in size and also comprises GAGE, SAGE, NY-ESO and XAGE, all of which are also located on the X chromosome[56–58]. The X-linked SSX proteins (SSX1–SSX5) have also been described as CT antigens[59]. Similar to the MAGE proteins, these proteins are normally expressed mainly in testis, but are overexpressed in some melanoma cell lines. In cancer cells, in which genome-wide demethylation is frequently observed, CT gene promoters are activated by demethylation. It has been demonstrated that loss of CT-antigen expression in some cancers is directly linked to the methylation status of the corresponding genes. Conversely, treatment of MAGE-negative tumour cell lines with 5-aza-2′-deoxycytidine induces MAGE expression[60]. Similarly, the expression of SSX proteins is also induced by demethylating agents[61]. Whether the epigenetic regulation of CT antigens is linked to X-chromosome status needs to be explored further. As downregulation of the expression of X-linked CT antigens has a key role in tumour-induced immune tolerance, such a relation between CT antigen expression and X-chromosome status would have significant consequences for the monitoring of cancer-vaccine trials using CT-derived immunogens.

X-chromosome genes that escape inactivation

As mentioned previously, X-chromosome inactivation is regulated regionally, and a fraction of the genes encoded on the inactive X chromosome escape silencing in normal cells. For example, Carrel *et al.* studied genes that escape X-chromosome inactivation in non-tumour cells and identified a set of 34 genes that are not silenced[62]. Genes escaping inactivation were shown to lack DNA methylation[63], histone methylation[64] and histone acetylation[65]. Many of the genes that escape X-chromosome inactivation have a Y-chromosome homologue, so there is a balanced dosage of gene products between males and females for these genes. For the genes with no Y-chromosome homologue (or when the Y-chromosome homologue is not expressed), this overexpression in females must be tolerated, and it seems that, in general, there is no need for dosage compensation for genes that escape inactivation. Dosage might not be important for these genes or, alternatively, higher levels of their expression might be important for a sex-specific function. It is also possible that in some cases the level of expression of the two alleles in female cells might be decreased to match that of the single allele in male cells. However, such an effect — which is known to operate as a generalized dosage-compensation mechanism in *Caenorhabditis elegans*[66] — is not documented in mammalian cells.

Some cancer-related genes are included among the genes that escape X-chromosome inactivation, most of which encode growth factors (TABLE 1). One of these is the gene encoding the gastrin-releasing peptide receptor (GRPR), which has been described as a growth factor for lung epithelial cells and the expression of which is associated with an increased risk of lung cancer in women[67]. The presence of two expressed copies of the *GRPR* gene in females might be a factor in the increased susceptibility of women to tobacco-induced lung cancer. Overexpression of genes that normally escape inactivation have also been implicated in the development of lymphomas through gene-expression studies. Using an X-chromosome-specific microarray

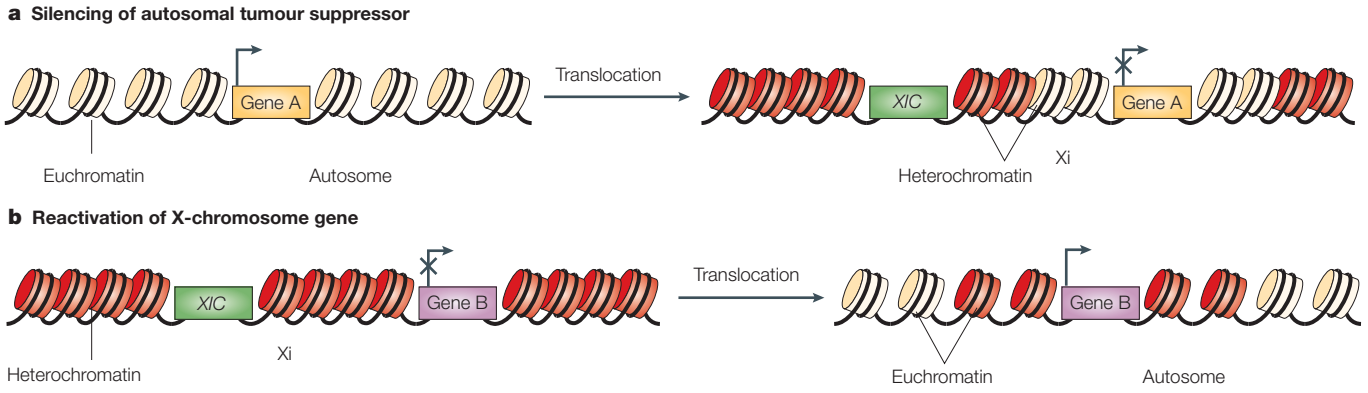


Figure 3 | **Tumorigenic effects of translocations involving the X chromosome.** The consequences of a translocation between an X chromosome and an autosome are variable. **a** | An autosomal tumour suppressor (gene A) can become inactivated if it is translocated to the inactive copy of the X chromosome. A gene that is normally located within transcriptionally active euchromatin on an autosome will become silenced if it moves by translocation to an area of the inactive X chromosome (Xi) in which it comes under the influence of the X-chromosome inactivation centre (*XIC*), which promotes the formation of transcriptionally silent heterochromatin and loss of gene expression. **b** | Conversely, an X-chromosome-linked gene (gene B) that is normally located on the Xi, and is therefore not expressed, can be reactivated by translocation to an autosome, with potential oncogenic consequences. In this situation, if the translocation separates the gene from the *XIC*, moving it to an area of transcriptionally active euchromatin on an autosome, the effects of heterochromatinization will be lost and the gene will be transcribed.

carrying cDNA fragments representing up to 1,317 X-chromosome genes, Sudbrak *et al.* compared gene-expression profiles in lymphoblastoid cell lines from normal males, females and individuals with supernumerary X chromosomes[68]. A total of 53 genes showed increased expression levels in cells with multiple X chromosomes, and many of these were found to escape X-chromosome inactivation in normal cells.

That some of the genes that escape inactivation might have tumour-suppressor activity was first indicated by a study of ovarian tumours[69]. These comprise a heterogeneous group of cancers, including ovarian borderline tumours and ovarian carcinoma, and it is not known whether these tumours are unrelated entities or whether borderline tumours are precursors to carcinoma. Cheng *et al.* assessed ALLELOTYPIC differences between ovarian tumours of different histological types, analysing 16 ovarian cystadenomas, 23 borderline ovarian tumours and 50 invasive ovarian carcinomas. Half of the borderline tumours showed LOH affecting the q arm of the X chromosome, most cases of which were due to interstitial deletions in X-chromosome genes. The alleles on the remaining regions of these chromosomes were assayed for methylation status, which showed that LOH affects the inactivated X chromosome in borderline tumours.

These observations indicate that genes escaping X-chromosome inactivation are targets for LOH and might therefore have tumour-suppressor activity. The same conclusion was reached by Piao *et al.*, who reported that loss of Xq25 — a region that contains a putative tumour-suppressor gene — correlates with an aggressive breast cancer phenotype and occurs preferentially on the inactive X chromosome[70]. Therefore, tumour-suppressor activity associated with X-linked genes might depend on their expression on the inactive X chromosome, with only the allele on this chromosome being active in these cases. This indicates either that these genes, like *XIST*, are expressed only from the inactive X chromosome, or that there is a dosage-control effect that acts asymmetrically on the two copies.

Translocations involving the X chromosome

Chromosomal translocations that bring together two unlinked segments of the genome can lead to inappropriate expression of a gene or can generate a new fusion gene that might have an oncogenic effect. Such events occur frequently in cancer cells as a result of intrinsic genetic instability. Cells carrying translocation events that lead to gene deregulation and confer a growth advantage are positively selected. When the X chromosome is involved in translocation events, specific types of gene-expression deregulation can occur that are not seen when autosomes alone participate in such events (FIG. 3). A translocation between the X chromosome and an autosome might physically remove an inactivated X-linked gene from the inactivation centre and lead to reactivation. Such somatic reactivation of a segment of the Xq chromosome in a t(X;15) translocation has been reported in a lymphoblastoid cell line from a patient with FANCONI ANAEMIA[71]. This indicates that reactivation might occur when part of the X chromosome is separated from *XIC*.

In addition, a translocation might silence an autosomal gene by placing it under the influence of *XIC*. X-chromosome inactivation can spread in *cis* through autosomal regions that are translocated onto the inactive X chromosome. This was first illustrated by Couturier *et al.* in a study of a constitutional t(X;21) translocation in a girl who was trisomic for chromosome 21, but who showed only a mildy abnormal phenotype of minor mental retardation and low SUPEROXIDE-DISMUTASE activity[72]. Trisomy for the whole of chromosome 21 — as was seen in this case —would be expected to produce a more severe phenotype. The

ALLELOTYPING
A technique used to identify the paternal and maternal alleles of a given gene based on polymorphisms.

FANCONI ANAEMIA
A rare disorder that is characterized by developmental abnormalities of the skeleton and other organs, defects in skin pigmentation, progressive failure of the bone marrow to replenish platelets and red and white blood cells, and susceptibility to acute myeloid leukaemia and squamous-cell carcinoma.

SUPEROXIDE DISMUTASE
An enzyme that is present in all aerobic organisms. It catalyses the conversion of highly reactive and destructive superoxide anion radicals, which are generated by the metabolism of the cell, into hydrogen peroxide.

authors demonstrated that the mildness of the phenotype probably reflected silencing of regions of chromosome 21 that were proximal to the X-chromosome sequences on the translocated chromosome.

Whether *XIST*, in addition to *XIC*, is necessary for inactivation of autosomal genes when they are placed under the influence of regions of the inactive X chromosome is a matter of debate. Insights into this have been provided recently by a study showing that silencing requires a conserved repeat sequence located at the 5′ end of *XIST*. Deletion of this element results in the production of *XIST* RNA that still associates with chromatin and spreads over the chromosome, but does not effect transcriptional repression[73]. This could explain the reports of constitutional X-chromosome/autosome translocations leading to incomplete and non-contiguous inactivation of autosomal genes[74].

In addition to those described above, similar observations have been reported for other translocations involving the inactive X chromosome and autosomes[75], and Sharp *et al.* have established that the silencing of autosomal genes can extend up to 45 Mb from the translocation point[76]. Tumour-suppressor genes located on autosomes might therefore undergo silencing as the result of such a mechanism. This phenomenon is illustrated in several reports of constitutional translocation involving the retinoblastoma gene (*RB*) on chromosome 13q. Retinoblastoma results from a two-hit event that is caused by biallelic loss of function of the *RB* tumour suppressor, which controls cell proliferation by regulating the activity of the transcription factor E2F1. Lack of functional RB results in constitutively active E2F, driving aberrant cell division. Translocation of chromosome 13q to the inactive X chromosome leads to functional silencing of RB as a result of the spreading of X-chromosome inactivation throughout the autosomal region[77], indicating the potential of such translocations to silence tumour-suppressor activity.

Defects in X-chromosome inactivation

Impaired X-chromosome inactivation. Any alteration in the complex pathway of X-chromosome inactivation is expected to perturb the fine-tuning of gene dosage for X-linked genes. Mutations in the coding region of *XIST* are rare, but mutations in the promoter have been reported[78,79]. Impairment of the maintenance of X-chromosome inactivation is illustrated in germ-cell tumours. *XIST* is expressed in tumours of this type (seminomas, non seminomas or spermatocytic seminomas) that carry a supernumerary X chromosome. However, even though *XIST* expression levels indicate that X-chromosome inactivation is functional, a different pattern of *AR* promoter methylation in differentiated and undifferentiated non-seminoma tumours has been reported[28]. *AR* methylation was observed in differentiated tumours but not in undifferentiated tumours, indicating that although initiation of X-chromosome inactivation seems to be operating in the undifferentiated tumours (as indicated by *XIST* accumulation), the maintenance of X-chromosome inactivation might be impaired. This might be a step towards oncogenesis, altering the dosage of *AR* and providing a selective growth advantage to undifferentiated tumours.

The BRCA1 paradigm. Hereditary early-onset breast and ovarian cancers are often linked to germline *BRCA1* or *BRCA2* mutations. *BRCA1* is an autosomal tumour-suppressor gene located at 17q21, and mutations in this gene account for 80–90% of cases of inherited ovarian cancer. *BRCA1* is thought to have a role in DNA repair, with mutations in this gene leading to an increase in genomic instability. However, the precise mechanisms involved in the development of breast and ovarian cancers in carriers of *BRCA1* mutations are not well understood.

Ganesan *et al.*[80] uncovered an unexpected link between *BRCA1* and X-chromosome inactivation, demonstrating that BRCA1 localizes to heterochromatin structures containing inactive X chromosomes, such as the XY bodies that are formed during meiosis in male mammals. Several lines of evidence support a model in which BRCA1 has a role in *XIST* RNA accumulation on the inactive X chromosome. BRCA1-deficient cells do express *XIST* RNA, but fail to assemble Barr bodies, and ectopic expression of wild-type BRCA1 in these cells restores specific *XIST* staining. Whereas sporadic breast and ovarian cancers, which do not carry mutations of *BRCA1*, show the hallmarks of X-chromosome inactivation — that is, nuclear expression of BRCA1, *XIST* staining and methylation at lysine 9 of histone H3 — tumours from women carrying *BRCA1* germline mutations do not show these features. Altogether, these data provide evidence that intact BRCA1 functioning is required for the maintenance of *XIST* localization to the inactivated X chromosome. Indeed, small interfering RNAs that suppress BRCA1 accumulation also suppress the accumulation of *XIST* on the inactive X chromosome.

Defects in X-chromosome inactivation might therefore contribute to oncogenesis caused by *BRCA1* mutation. Jazaeri *et al.* have reported microarray data showing that the expression of a set of genes that includes an unexpectedly large fraction of X-linked genes can distinguish between BRCA1-deficient and sporadic ovarian carcinomas[81]. Interestingly, the transcription of these X-linked genes is upregulated in BRCA1-deficient tumours, supporting the hypothesis that failure of X-chromosome inactivation is involved in tumorigenesis. However, as pointed out by Jazaeri *et al.*, the link between the lack of BRCA1 and relaxation of X-chromosome silencing might alternatively reflect a selective pressure exerted during ovarian carcinogenesis for overexpression of X-linked genes that are required for ovarian development and function[81].

BRCA1 mutations have also been associated with X-chromosome LOH. For example, it was reported that LOH at Xp22.2–22.3 is associated with germline mutation of *BRCA1* in ovarian cancer[38]. LOH of this region is twice as frequent in carriers of *BRCA1* germline mutations and mostly affects the active allele, leading to the hypothesis that there is a tumour-suppressor gene locus at Xp22.2–22.3 and that LOH of this gene is involved in *BRCA1*-linked carcinogenesis.

Skewed X-chromosome inactivation. As described above, X-chromosome inactivation is usually a random process, so that, in females, the paternal X chromosome is inactivated in 50% of cells and the maternal copy is inactivated in the other 50%. However, skewed (non-random) X-chromosome inactivation has been observed in some tissues in non-disease conditions and is also expected to perturb the phenotypic expression of oncogenic events. Relaxation of epigenetic regulation is not uncommon during ageing and leads to reactivation of transcriptionally silent X-linked genes and/or skewed X-chromosome inactivation[80,81]. As a consequence of skewing, the phenotype associated with an oncogenic mutation — tumour growth — might either remain silent if inactivation exclusively affects the X chromosome that carries the mutation, or might be more pronounced if it affects the wild-type chromosome. More importantly, mutational events that potentially activate an oncogene on the silenced copy of the X chromosome in females will remain silent until they become uncovered following the relaxation of epigenetic regulation that takes place during ageing. Therefore, X-chromosome inactivation transiently protects females from the deleterious consequences of those oncogenic events that affect the inactive X chromosome. This is expected to translate into delayed onset for some types of cancer in females compared with males, although this has not yet been demonstrated.

Skewed X-chromosome inactivation has been shown to account for some cases of X-linked recessive disorders in females. Female carriers of some of these conditions show skewed X-chromosome inactivation by chance, and in cases where most of the cells express the mutated gene as a result of skewing, this can cause manifestation of the disease. For example, this is seen in females with Duchenne muscular dystrophy, which is caused by mutations in the dystrophin gene located on the X chromosome. In one study of another disorder of this type — WISKOTT–ALDRICH SYNDROME, which is associated with increased susceptibility to leukaemia — skewed X-chromosome inactivation was seen in a girl with the condition[82]. This patient was heterozygous for a mutation in exon 4 of the *WASP* gene located at Xp11 and showed expression of the mutant allele from the active X chromosome only. Therefore, all of the active X chromosomes in this patient carried the mutation. Skewed X-chromosome inactivation has also been observed in healed skin tissues and lymphocytes of females affected with INCONTINENTIA PIGMENTI, a disease associated with X-linked dominant mutation of *NEMO* (a gene involved in NF-κB-mediated inhibition of apoptosis). This distortion reflects the positive selection of cells carrying the mutation on the inactive X chromosome; these cells survive, whereas those carrying the mutation on the active X chromosome undergo cell death due to the absence of anti-apoptotic NF-κB activity[25,83,84]. A similar positive-selection mechanism might lead to the growth of tumour cells that are heterozygous for a mutation in an X-linked tumour-suppressor gene, providing that the wild-type allele is functionally silenced as the result of X-chromosome inactivation.

A possible relationship between skewed X-chromosome inactivation and cancer has gained support from studies of leukaemias and ovarian cancers. In a stem-cell line with the karyotype 45,X,–X,t(8;21) (that is, lacking one copy of the X chromosome in addition to a translocation between chromosomes 8 and 21), which was derived from a female patient with acute myeloid leukaemia (AML), the remaining X chromosome was found to be the active copy. This indicated that in patients with AML with the acquired t(8;21) translocation, the loss of the inactive X chromosome in females and the Y chromosome in males (which occurs in nearly 50% of patients) provides a selective advantage to the stem-cell line[85]. In addition, Buller *et al.* reported that 53% of patients with invasive ovarian cancer showed non-random X-chromosome inactivation, compared with 28% of patients with borderline tumours and 33% of normal volunteers[86,87]. Interestingly, they observed that among 11 cases of ovarian cancer in *BRCA1* germline mutation carriers, 9 displayed non-random X-chromosome inactivation. They suggested that their observations provide evidence for the presence of a tumour-suppressor gene on the X chromosome acting as a genetic modifier of *BRCA1* PENETRANCE. Kristiansen *et al.*[88] also reported a high frequency of skewed X-chromosome inactivation in young patients with breast cancer. Although these patients were not genotyped as *BRCA1*/*BRCA2* mutation carriers, considering the selection for early onset of disease, a significant proportion of them are likely to be carriers.

Conclusions and future perspectives

X-chromosome unisomy provides a unique genetic context for the expression of the common genetic events that cause cancer — that is, oncogene activation and tumour-suppressor inactivation. Because females have two X chromosomes, they might be expected to be less susceptible to mutations in tumour-suppressor genes. However, the lack of significant difference in overall risk of cancer between males and females — excluding the risks linked to gender-specific organs and environmental factors — demonstrates that genetic unisomy in males and functional unisomy in females are operationally equivalent, and that X-chromosome inactivation operates efficiently. Nevertheless, as suggested earlier, the silenced copy of the X chromosome might 'hide' oncogenic events and, therefore, make female cells resistant to early-onset cancer-promoting events, until these events become unmasked during the relaxation of silencing that takes place during the ageing process[89]. This hypothesis predicts that an excess of mutations should be observed on X-linked genes in ageing female cells, although further studies are necessary to test whether this is the case.

The essential lesson that can be drawn from the studies discussed in this review is that gene dosage seems to be a central mechanism in the oncogenic processes that are linked to X-chromosome alterations. X-chromosome gains, gains in active versus inactive X chromosomes,

WISKOTT–ALDRICH SYNDROME
An X-linked genetic disorder that almost always affects males and is characterized by thrombocytopaenia, eczema, melena and susceptibility to bacterial infections because of severe immunodeficiency.

INCONTINENTIA PIGMENTI
An inherited hypopigmented skin lesion that shows a so-called 'marble-cake' pattern, which is variably associated with epidermal nevi, alopecia, and ocular, skeletal and neural abnormalities.

PENETRANCE
The frequency with which individuals who carry a given mutation show associated phenotypic manifestations. If the penetrance of a disease allele is 100%, then all individuals carrying that allele will express the associated phenotype.

escape from inactivation, reactivation of X-chromosome genes and impaired X-chromosome inactivation are all expected to drive gene overexpression. However, there is very little information about the identity of the genes that are targeted for overexpression, their levels of expression and, therefore, the functional consequences of such increased dosages of their gene products. The actual molecular basis for the oncogenic effects of doubling the copy number of the genes that are affected is also unclear. Careful expression profiling of X-linked genes in tumour cells should help to understand how X-chromosome-related events lead to oncogenesis. This type of approach has already yielded promising results. For instance, the fact that *XIST* binding of the inactive X chromosome[80] and upregulation of a set of X-linked genes[81] can both distinguish BRCA1-deficient and sporadic ovarian carcinomas has provided new insights on the role of BRCA1 in the maintenance of heterochromatinization on the inactive X chromosome.

An increased understanding of the expression patterns of X-linked genes in cancer cells is also likely to provide useful information for diagnostic and prognostic purposes. For example, characterization of *XIST* expression in a panel of female cancer cell lines demonstrated a significant correlation with paclitaxel sensitivity. So far, this observation remains unique and has no obvious biological rationale, but has clear implications for predicting responses to the treatment for this type of cancer. Along the same lines, it is noteworthy that the *XIST* minimal promoter — which is silenced by complete methylation in male somatic cells — becomes hypomethylated, and therefore probably active, in TGCTs. Identification of an unmethylated fragment of *XIST* could therefore potentially be used as a diagnostic tool for this type of tumour. Indeed, such fragments have been detected in the plasma of men affected with TGCTs[90] Whether such approaches are useful for other cancers will require further exploration.

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Competing interests statement

The authors declare no competing financial interests.

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