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X Chromosome Inactivation and Autoimmunity

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Abstract Autoimmune diseases appear to have multiple contributing factors including genetics, epigenetics, environmental factors, and aging. The predominance of females among patients with autoimmune diseases suggests possible involvement of the X chromosome and X chromosome inactivation. X chromosome inactivation is an epigenetic event resulting in multiple levels of control for modulation of the expression of X-linked genes in normal female cells such that there remains only one active X chromosome in the cell. The extent of this control is unique among the chromosomes and has the potential for problems when regulation is disrupted. Here we discuss the X chromosome inactivation process and how the X chromosome and X chromosome inactivation may be involved in development of autoimmune disorders.

Keywords X chromosome · X inactivation · Autoimmunity · Polyamines

Abbreviations

dcSAM	Decarboxylated S-adenosylmethionine
EBV	Epstein–Barr virus
LINE	Long interspersed nuclear element
MS	Multiple sclerosis
PAD	Peptidyl arginine deiminase
PAR1, PAR2	Pseudo-autosomal regions of the X chromosome
SAF	Scaffold attachment factor
SAM	S-adenosylmethionine, the cellular methyl group donor
SAMDC	SAM decarboxylase, a key, initial enzyme in polyamine synthesis
SAT1	Spermidine/spermine-N1-acetyltransferase, an enzyme in polyamine recycling
SMS	Spermine synthase, an enzyme in polyamine synthesis
TSIX	An anti-sense gene to the XIST gene, involved in initiation of XCI
Xa	The active X chromosome
XAR	X-added region
XCI	X chromosome inactivation, epigenetic silencing of X chromosomes
XCR	X-conserved region
Xi	The inactive X chromosome
XIC	X-inactivation center, locus of genes involved in initiating XCI
XIST	X-inactivation specific transcript, a key gene in initiating XCI
X-CGD	X-linked chronic granulomatous disease
Xp	X chromosome short arm
Xq	X chromosome long arm

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Introduction

Female cells have two X chromosomes whereas male cells have only one. Most X-linked genes are not sex-specific

and, therefore, should have equal expression in males and females. Female mammalian cells inactivate one X chromosome to achieve equivalency, in a process referred to as X chromosome inactivation (XCI), a means of dosage compensation. XCI is a major epigenetic event involving multiple levels of gene silencing biomarkers and differences in temporal and spatial treatment of the inactive X (Xi) relative to other chromosomes. Inability to establish or maintain XCI can lead to pathological states. For example, in females with tiny X rings, where one X has broken at two sites and rejoined into a ring, the ring may lack key genes for XCI. The cell may then over-express genes from the ring and active X (Xa). These females can have Turner syndrome features (short stature and ovarian abnormalities) and severe mental retardation, developmental delay, fused digits, dysmorphic facial features, and heart defects [1].

X-linked dosage compensation can occur via different methods across species. In the nematode, *Caenorhabditis elegans*, females achieve dosage compensation by reducing expression from both X chromosomes by 50%, to equal expression of the single X in the male [2]. In the fruit fly, *Drosophila melanogaster*, dosage compensation is achieved by doubling expression of the single X in males to match expression in females [2]. Here we focus on the method used in mammals, in which only one X is kept active in female cells and has approximately the same expression as seen in males.

Autoimmune diseases occur in 3–5% of the population with females being in the majority [3]. Even among males, those with Klinefelter's syndrome (XXY), have an increased risk relative to normal males [4]. In multiple sclerosis (MS), men transmit the disease more often to their children than do women [5]. These reports suggest involvement of the X in autoimmune diseases.

The X chromosome

The X and Y chromosomes are believed to have originated from a common ancestral autosome. Since then, the Y chromosome has declined in size (to 60 mb) and in genes (approximately 100 genes). On the other hand, the X chromosome has incorporated genes from autosomes so that it is now 155 mb with 1,098 genes and 700 pseudogenes [6]. However, in the distal regions of X and Y arms there remain homologous regions (see Fig. 1) that have sufficient commonality to recombine during meiosis, leading to rare translocations. The X chromosome accounts for 5% of active genes in humans and this has been consistent across many mammalian genomes [7]. However, despite its size and percentage of the genome's total active genes, the X chromosome has a relatively low density of genes [6]. Table 1 lists interesting features of the X chromosome in comparison to other chromosomes.

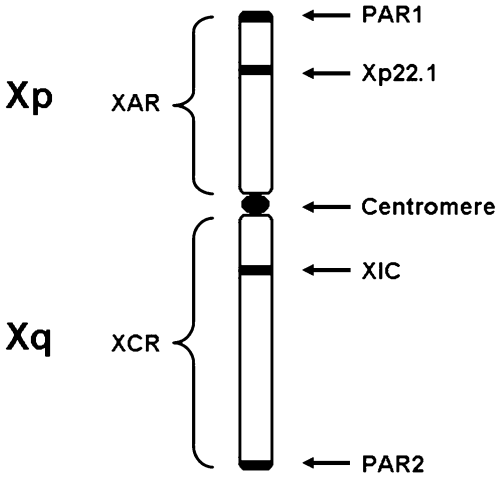

Fig. 1 X chromosome regions. Regions in the X chromosome are: *Xp* (short arm), *Xq* (long arm), *PAR* (pseudo-autosomal region), *XAR* (X-added region), *XCR* (X-conserved region), and *XIC* (X-inactivation center)

X-linked fragile sites and genes neighboring them, appear to be important in the stability of the X. Fragile sites can stretch for hundreds of kilobases and are characterized by increased frequency and sensitivity to DNA damage and alterations. Alterations in fragile sites can include plasmid DNA integration; translocations; rearrangements; sister chromatid exchanges; amplifications; plasmid insertions; deletions; and even gaps [8]. Fragile sites are considered to be 'hotspots' for these types of damage and are frequent integration sites for viral sequences, such as human papillomavirus type 16 [9]. Common fragile sites are late replicating, which means that, even if damage is repaired correctly, these sections of chromatin are more vulnerable to destabilization since epigenetic markers may be disrupted during the damage and repair process [10]. There are 84 known common fragile sites in the human genome, and 80% of lesions in lymphocytes can be attributed to gaps and breaks in only 20 of these sites [10]. One common fragile site is FRAXB at Xp22.3. FRAXB is A-T rich, has a high content of repetitive elements, and neighbors the toll-like receptor 5a gene [11]. This proximity of a fragile site to an immune-related gene is of interest with regards to autoimmunity. Other fragile sites exist on the X, including the rare fragile site, FRAXA, associated with fragile X-linked mental retardation [10].

X chromosome inactivation and the X-inactivation center

Females have a high probability of receiving at least one viable copy of each X-linked gene since they inherit two X chromosomes. Males do not have an alternative for X-linked genes. Therefore, males are vulnerable to X-linked

Table 1 X chromosome features

Feature	Details
G + C content	39% in the X chromosome versus 41% for the genome average. However, PAR1 is 48% G + C.
CpG islands	5.25 per mb in the X chromosome versus ~10 per mb for the genome average. However, 49% of genes on X have CpG islands.
Interspersed repeat elements	56% in the X chromosome versus 45% for the genome average.
Alu SINEs	8.23% in the X chromosome versus 10.60% for the genome average. However, PAR1 in the X is 28.88% Alu.
LINEs	29% in the X chromosome versus 17% for the genome average. However, the LINE-1 content in the X goes from 33.5% in XCR down to 6.97% in PAR1.
Heterozygosity	The X chromosome is 57% heterozygous relative to the genome average.
SNPs	153,146 SNPs in the X, 901 of which are non-synonymous in protein coding.
Base pairs	155 mb, making the X chromosome the eighth largest in the genome.
Genes	The X chromosome has 1,098 genes in 155 mb, which is lower density than the genome average.
Pseudo-genes	The X chromosome has 700 pseudo-genes in 155 mb, which is lower density than the genome average.
XAR	X-added region, contains DNA sequences incorporated since divergence of the X and Y chromosomes from a common ancestral autosome.
XCR	X-conserved region, contains DNA sequences believed to reflect the original ancestral autosome.

Based on data in the paper by Ross et al. [6]

genetic diseases, such as hemophilia, color blindness, X-linked chronic granulomatous disease, and X-linked autoimmunity-allergic disregulation syndrome [12]. Females are carriers of these disorders, avoiding the consequences for the most part. However, females can have problems managing X-linked dosage compensation.

In most cases, the choice of which X chromosome to inactivate, the paternally derived or the maternally derived, is a random choice made early in development of the embryo. Both X chromosomes are active initially. However, at the late blastocyst stage, as pluripotent cells are differentiating, the decision is made in each cell as to which X to keep active. After that, all cells originating from that initial cell will maintain the same parentally derived XCI pattern. If, for example, a precursor liver cell chose to keep the maternally derived X active, then years later, cells in that area of the liver will have the maternally derived X active and the paternally derived X will be inactive. The adult is then a mosaic of areas with the maternally derived X inactivated next to areas with the paternally derived X inactivated.

Studies on mice have given us much of our knowledge on XCI, such as events occurring when XCI initiates. Conservation of X chromosome features allows us to apply this knowledge to humans. Through study of X-linked translocations and deletions, we have identified the X-inactivation center in mice (Xic) and humans (XIC) at Xq13 [13]. XCI initiates from the XIC and spreads to contiguous chromatin. Genes closer to the XIC have a greater probability of inactivation, whereas genes further away have a greater chance of escaping inactivation, particularly in pseudo-autosomal regions. The net result is that most genes on the Xi are inactivated. Carrel et al. reported 34 of 224 X-linked genes (15%) studied still showed some activity from the Xi after XCI was established [14]. They estimated 35% of Xp genes and 5% of Xq genes were expressed to some extent. So we can think of 85% of genes being inactive in a typical Xi, but the number of inactive genes on the Xi can vary from cell to cell and the extent of inactivation of individual genes can vary from female to female [15]. In addition, we see changes in X-linked expression with age, suggesting age-related loss of dosage compensation.

The X chromosome has a higher content than other chromosomes of long interspersed nuclear elements (LINEs), especially LINE-1. The LINE-1 distribution shows greater density in the long arm (Xq), going to lower density in the short arm (Xp). XCI initiates from the X-inactivation center at Xq13 in the long arm. Mary Lyon, who first described XCI, [16] has proposed a role for LINE1 elements in the spread of XCI, from the XIC since the LINE-1 distribution parallels the spread from the XIC and the intensity of gene silencing in the Xi [17].

The X-inactivation center and macroRNAs

The human XIC at Xq13 is a locus of overlapping sense and anti-sense genes that generate macroRNAs that interact in XCI initiation. The main gene of interest is the X-inactivation specific transcript, or simply XIST in humans (Xist in mice). The XIST gene is approximately 35 kb (Xist is 23 kb) and yields alternate spliced transcripts of up to 19.3 kb that localize in the nucleus and coat the future Xi

[18]. The Xist transcript remains in the nucleus and does not appear to code for protein.

Overlapping the Xist gene and running anti-sense to it is the Tsix gene. Before XCI initiates, mouse embryonic cells express Xist and Tsix from both X chromosomes, with expression of Tsix being greater. Neither Xist nor Tsix RNA transcripts persist at this time. However, Tsix expression leads to a change in chromatin shared with Xist. This chromatin becomes enriched in histone H3 methylated at lysine 4, an active gene marker [19]. This chromatin change allows for efficient expression of Xist and Tsix with DNA looping in the sequences [20]. This clears each X chromosome of any pre-imprinted bias towards inactivation of one X over the other.

Choosing which X chromosome to inactivate

In order to make a random choice as to which X chromosome to inactivate, there is co-localization of the X chromosomes, with pairing of the Xic loci [21]. Then, several genes within the Xic express non-coding cis-acting macroRNAs that interact within the Xic leading to the Xi and Xa choice [22]. DXPas34 is located 3' to Xist and appears to be required for 'counting' of X chromosomes so there is adherence to the 'N-1' rule, i.e. all X chromosomes but one are inactivated (Fig. 2). Xite, a small gene upstream of Tsix modulates expression of Tsix. Differential methylation in Xite and in CTCF binding sites in Tsix act to alter expression of Tsix simultaneously in the paired X chromosomes [23, 24]. This suggests a stoichiometric means of choosing the future Xa as CTCF proteins bind sites in Tsix, reaching a critical mass on one X before the other. In the future Xa, Tsix expression persists longer. In the future Xi, Tsix expression drops as its gene loses transcriptional competence. Now in the Xi, Xist expression increases, taking advantage of what remains of transcriptional competence established earlier. Xist transcripts now persist and begin to coat the Xi. Xist transcripts persist in the Xi except during mitosis when they are temporarily displaced.

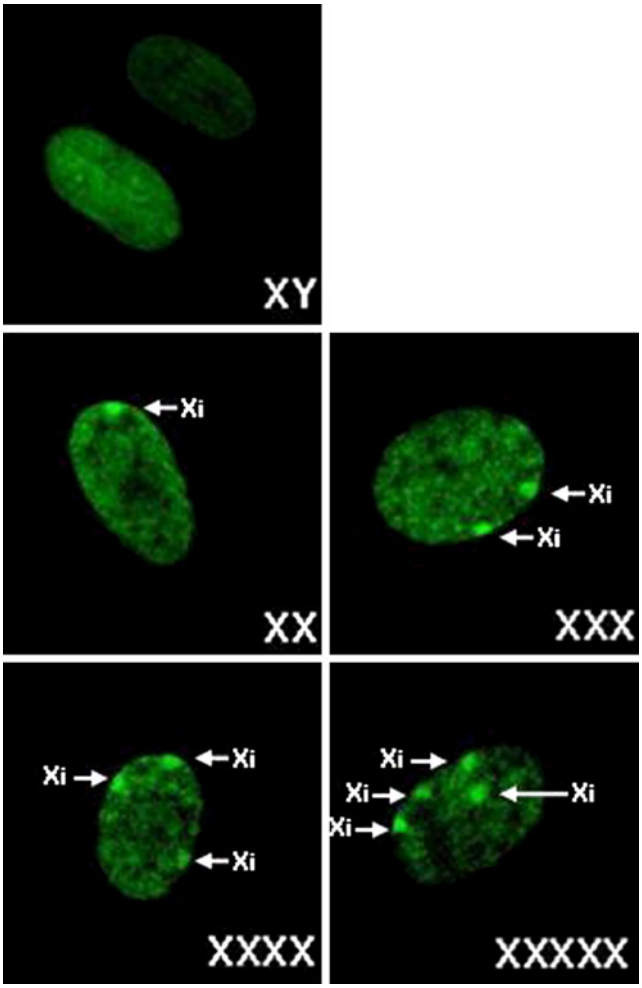

Fig. 2 Confocal images of human fibroblasts showing nuclei stained with anti-H1S-2 histone antibodies: the 'N-1'rule. Human fibroblasts differing in the number of X chromosomes were incubated with rabbit antibodies targeting human histone H1 subtype 2 and then incubated with anti-rabbit IgG FITC-conjugated antibodies. These panels show anti-H1S-2 immunofluorescence of human fibroblasts with increasing numbers of X chromosomes: (46, XY), (46,XX), (47,XXX), (48, XXXX), (49,XXXXX). Xi appears as a brightly stained heterochromatic body. DAPI staining (not shown) concurs with the Xi location. The 'N-1' rule means that, for N X chromosomes, there are N-1 inactive X chromosomes in the cell. Courtesy of Dr. Thomas P. Yang, University of Florida, Gainesville, FL and Dr. Missag H. Parseghian, Peregrine Pharmaceuticals, Inc., Tustin, CA

Establishing epigenetic control on the inactive X chromosome

Expression of Xist from the chosen X is sufficient to initiate silencing of the chromosome, but this ability appears to be confined to early development [25]. Subsequent expression of Xist after differentiation does not induce silencing and does not appear necessary for maintenance of silencing, since other epigenetic markers can pass the XCI pattern to daughter cells [26]. Xist transcripts bind further from the XIC along the Xi as the inactivation process spreads. The XIST RNA then appears to recruit proteins that will add additional epigenetic markers. Among these markers, DNA methylation in 5′ promoter regions of genes is an immediate reaction to XIST RNA binding. Methylation of LINE-1 by DNMT3B occurs concurrently with the spread of silencing [27]. Another early Xi epigenetic marker is dimethylation of histone H3 at lysine 9 [28]. Later, trimethylation of H3 at lysine 27 and monomethylation of H3 at lysine 20 occur [29]. These three markers signify gene silencing. There is little methylation of H3 at lysine 4, which typically denotes active genes [28].

Another early Xi marker is an enrichment of macroH2A, (variants macroH2A1.2 and macroH2A2). The distributions of these variants, however, vary by cell types, suggesting developmental determinants in their incorporation [30]. The N-terminal one third of macroH2A has 64% identity with histone H2A but the rest of macroH2A is unique and contains domains that direct macroH2A to the Xi [31, 32]. Although macroH2A is expressed in most cells, including in males, it is concentrated in the Xi in differentiated female cells with an estimate of one molecule for every 30 nucleosomes, suggesting a role in transcriptional repression [30].

Other epigenetic markers of the Xi include hypoacetylation of histone H4; ubiquitination of lysine 119 of histone H2A; and the inclusion of histone H1. On the other hand, the silenced sections of the Xi exclude histone H2A.bbd (Barr body deficient) [33] and are low in RNA polymerase II. A review of Xi epigenetic markers and their order has recently been published [29]. The Xi is also enriched with scaffold attachment factors (SAF), which fractionate with Xist RNA in nuclear matrix preparations, suggesting scaffold attachment has a role in organizing the Xi into higher-order chromatin [34].

The Barr body

The physical appearance of the Xi was first noted by Barr and Carr who reported a correlation between distinct dense perinuclear heterochromatic bodies in interphase nuclei and the number of X chromosomes in a range of human karyotypes from (45,X0), (46,XY), (46,XX), to mosaics of (45,X0/47,XXX) and (46,XY/48,XXXY) [35]. Since then, Xi is also called the Barr body, and can be seen with staining (Fig. 2). The Xi is found near the nuclear membrane and away from other chromosomes, which tend to be more euchromatic and spread out during interphase. The Xi and Xa occupy similar volumes, even though the Xi has a condensed ultrastructure [36]. The Xi, under close scrutiny, shows condensed fibers with space between them, giving the Xi an appearance differing from both euchromatin and typical heterochromatin. This suggests interaction of Xist RNA and SAF are involved in organizing these fibers with the nuclear scaffolding and matrices. The Xi outer portion is gene-rich while the inner portion contains silenced and non-gene sequences [37]. Following inactivation, the Xi switches to late replication in S phase, later than other chromosomes. In addition, 80–90% of the Xi takes on a perinucleolar localization during mid-to-late S phase, a pattern not seen with other chromosomes. This association with the nucleolus is believed to be required for maintenance of the Xi's epigenetic state [38]. We should also remember that fragile sites, including X-linked fragile sites, are late replicating, suggesting significant vulnerability in the Xi to maintain its epigenetic markers through generations of cell cycling. It must effect DNA repairs, and reestablish the local epigenetic state in and around S phase since it is condensed and localized to the nuclear periphery during much of the cell cycle, reducing accessibility to repair. This vulnerability of the Xi could lead to loss of the Xi due to nucleases or reactivation of the Xi through loss of epigenetic markers. A study on the Xi status in Klinefelter's syndrome cells and female-derived cancer cells found that, in most situations where there was loss of the Xi, there was a gain in active X chromosomes due to multiplication of the original Xa [39]. In a few cancer cells there was gain of X-linked expression due to Xi reactivation. Dysfunction of BRCA1, a protein that colocalizes with XIST and is involved in DNA repair, can lead to failure of X inactivation. In addition, defects in the Xi in BRCA1-deficient breast and ovarian cancer cells can occur [40, 41].

Autoimmunity and skewing of the X-inactivation choice

Skewing of XCI, also called non-random X inactivation, is defined as a bias towards inactivating either the maternally derived X or the paternally derived X. Interest in skewing of XCI relates to the possibility that one or more genes on one of the X chromosomes may have genetic defects, leading to either a non-functional gene, altered expression levels of the gene, or a protein that has altered functionality due to mutations. In males, if the gene is important, the defective X would likely lead to a non-viable embryo. The female embryo may develop since sufficient cells would inactivate the defective X an expected 50% of the time. We typically define skewing as ≥75%. For example, choosing to inactivate the maternally derived X in 75% or more of cells gives skewed X inactivation towards the maternally derived X [42]. Extreme skewing is a bias of ≥90%. Testing for skewing is typically done using peripheral blood cells. This can be informative but does not tell us what is going on in other tissue types, which may be more important. Skewing of XCI can occur early in development and it can also occur over time as one parentally derived X chromosome leads to selective cell death due to problems with the Xa genes or with maintaining XCI [43]. Even with these caveats, skewing coincides with some diseases, such as dyskeratosis congenital, Rett syndrome, and X-linked chronic granulomatous disease [44–46].

As far as XCI skewing in autoimmune diseases, Stewart hypothesized it could be a factor in development of autoimmune diseases [47]. One scenario he defined was a biased presentation of self-antigens by antigen-presenting cells during thymic development of tolerance and negative selection of auto-reactive T cells. If self-antigens from the

inactivated parentally derived X chromosome were not represented, then negative selection of auto-reactive T cells towards those self-antigens could not occur. Countering Stewart's hypothesis, Chitnis et al. did not find evidence for a bias in XCI, but they noted there may be a local bias in XCI in the thymus, which skews development of tolerance for self-antigens [48]. We should also consider that self-antigen presentation in the development of tolerance in the periphery may be skewed. Knudsen et al. also reported they did not find significant differences in XCI patterns in multiple sclerosis patients compared to normal subjects [49]. However, they noted a possible increase in skewing when comparing remitting cases to progressive cases. A few recent reports link skewed XCI to autoimmune diseases, such as scleroderma and autoimmune thyroid disease [50, 51]. In general though, we do not have evidence linking skewed XCI to the majority of autoimmune diseases.

Autoimmunity and X chromosome vulnerability

The vulnerability of the X chromosome and XCI could also play a part in autoimmune diseases. In autoimmune diseases, we find cases with chromosomal abnormalities, many related to the X chromosome. Although many of the abnormalities are genetic in nature at the start, such as gene duplications, they can create difficulties for the cell to establish or maintain XCI, leading to problems of loss of dosage compensation of X-linked genes. Control of dosage compensation is the fundamental purpose of XCI. The loss of dosage compensation may be restricted to a few X-linked genes at the abnormality site. Alternatively, the loss may be more extensive, for example, if the abnormality halts the spread of XCI from the XIC, more distant X-linked genes may be blocked from the spread of inactivation by the abnormality.

Table 2 lists some of the associations between X chromosome abnormalities and autoimmune diseases. For example, Chagnon et al. reported a case of severe systemic lupus erythematosus (SLE) in an XX male who had an Xp22.33;Yp11.2 translocation that resulted in duplication of PAR1 genes and a partial trisomy of some genes [51]. They found 12 genes in the translocation, most interesting were the gene for the alpha subunit of the interleukin-3 receptor (IL-3 being a growth factor for proliferation and differentiation of hematopoietic stem cells) and CD99 (a transmembrane protein involved in adhesion and apoptosis of T cells).

Another interesting insight on the vulnerability of the X in relation to autoimmune diseases comes from another disease not normally considered to have autoimmune ramifications. X-linked chronic granulomatous disease (X-CGD), is an X-linked recessive disease in which phagocytes in X-CGD sufferers are not able to generate superoxide anions (O_2^-) that are converted to hydroxyl radicals, hydrogen peroxide, and other radicals that serve as oxidants to destroy bacterial material ingested by the phagocytes. X-CGD patients suffer from continual infections since their bodies cannot clear the bacteria. The gene most frequently involved in X-CGD is the cytochrome B β subunit gene (CYBB) located at Xp21.2.

Table 2 X chromosome abnormalities and autoimmune diseases

Site	Problem	Autoimmune Manifestation	References
Xp22.33	Xp22.33;Yp11.2 translocation causing triplication of genes.	Severe lupus	[51]
Xq26	Demethylation of CD40LG on the Xi in T cells in women, leading to over-expression of CD40LG in CD4+ T cells.	Lupus	[52]
X, specific site not identified	Skewed XCI.	Autoimmune thyroiditis	[53]
X, specific site not identified	Skewed XCI.	Scleroderma	[50]
Xp11.23	Mutated JM2 fork head-related protein (a.k.a. Foxp3, scurfin).	X-linked autoimmunity-allergic disregulation syndrome	[12]
X, specific site not identified	50% of MS patients versus 2% of control showed chromosomal abnormalities in peripheral blood cells. Half of MS abnormalities were X-related.	Multiple sclerosis	[54]
Xp21.2	Various defects (ex. duplication) in cytochrome B β subunit in sufferers and carriers of X-linked chronic granulomatous disease.	Lupus-like symptoms in some female carriers and some male patients with X-CGD.	[55;56]
X, specific site not identified	14-fold higher incidence of lupus in males with Klinefelter's syndrome (XXY) compared to normal males.	Lupus	[4]

This table lists some X-linked abnormalities associated with autoimmune diseases

Since it is X-linked recessive, females are usually carriers and male offspring are sufferers. The relevance of X-CGD to autoimmune diseases comes from a number of reports on SLE-like symptoms appearing in X-CGD patients and mothers who carry the X-CGD trait [55, 56]. This suggests that aberrations at Xp21.2 set up the possibility for over-expression of genes from that region and that over-expression is somehow involved in the subsequent autoimmune symptoms. It demonstrates the difficulties in establishing and maintaining epigenetic control when there are underlying abnormalities in the DNA.

Other diseases can give insights regarding the X's vulnerability. In sporadic basal-like cancers (BLC), a class of human breast cancer, there are frequent X chromosome abnormalities encountered, such as multiple active copies of Xp in cells negative for XIST and methylation at histone H3 lysine 27. There was over-expression of X-linked genes in ~50% of the BLC cells and two-thirds of that over-expression originated from Xp22 and Xq26–28 [57]. Those authors hypothesized that dysregulation of expression of some Xp22 genes is important in the pathogenesis of BLC, and they noted that other researchers looking at tumors with BRCA1 mutations had identified over-expression of some of the same X-linked genes as found by Richardson et al. [57, 58]. Parallels in autoimmune diseases and cancers have been discussed previously [59].

X-linked polyamine genes and autoimmune diseases

A key aspect of some autoimmune diseases, such as lupus, is abnormal methylation patterns. As a final topic for our discussion, we will touch on a hypothesis that relates loss of X-linked dosage compensation to the alterations in methylation seen in autoimmune diseases.

Elevated levels of polyamines are found in autoimmune patients [60]. The polyamines are ubiquitous small flexible cations that are essential for cell growth and proliferation [61, 62]. Some putative functions and interactions attributed to polyamines of interest with regards to autoimmune diseases are stimulating histone acetylation [63]; increasing blood–brain and blood–nerve barrier permeability [64]; modulating ion channels [65]; modulating nuclear receptor interactions, such as increasing the affinity of estrogen receptor for DNA [66, 67]; modulating DNA repair [68]; and stabilizing Z-DNA [69]. Because of the important interactions of polyamines, synthesis and recycling of polyamines are some of the most tightly controlled cellular processes (Fig. 3). The main polyamines of interest are spermidine (+3 charge, ~11 Å long) and spermine (+4 charge, ~15 Å long), and the precursor, putrescine (+2 charge, ~6 Å long). The charge distribution and length of polyamines give them the potential for unique interactions,

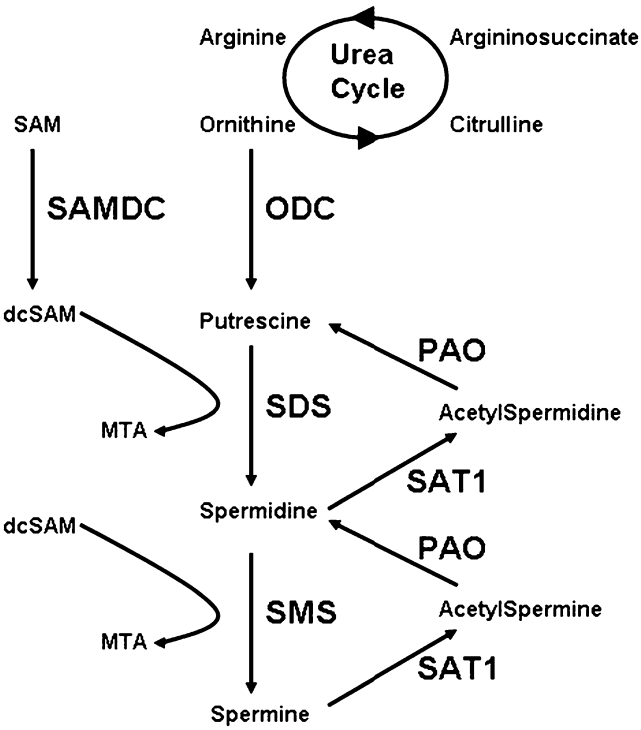

Fig. 3 Polyamine synthesis and recycling. Enzymes are: S-adenosylmethionine decarboxylase (*SAMDC*), ornithine decarboxylase (*ODC*), spermidine synthase (*SDS*), spermine synthase (*SMS*), spermidine/spermine-N1-acetyltransferase (*SAT1*), and polyamine oxidase (*PAO*). Other molecules are: S-adenosylmethionine (*SAM*), decarboxylated S-adenosylmethionine (*dcSAM*), and methylthioadenosine (*MTA*)

such as connecting anionic points in intramolecular and intermolecular complexes, (ex. aggregating transmembrane phospho-proteins).

A hypothesis has been proposed that explains some autoimmune diseases as occurring due to loss of dosage compensation of X-linked polyamine genes at Xp22.1, which impact intracellular methylation [70–72]. The two genes of interest are spermine synthase (SMS), an enzyme involved in synthesizing spermine from spermidine; and spermidine/spermine-N1-acetyltransferase (SAT1), an enzyme involved in recycling spermine and spermidine. Over-expression of SMS would lead to increased levels of spermine, particularly when unbound spermidine is available, as occurs with cell cycling and stress response (ex. UV light or viral activity). Over-expression of SAT1 increases recycling of polyamines. Increased polyamine synthesis uses decarboxylated S-adenosylmethionine (dcSAM), created from S-adenosylmethionine (SAM) by SAMDC. The resulting decrease in SAM, the cell's methyl donor, would hamper methylation in the cell. This could account for the aberrant DNA methylation seen in some autoimmune diseases.

Besides epigenetic control of gene expression, methylation is also a means of controlling intracellular signaling and protein trafficking [73]. For example, we mentioned

SAF as being involved with Xist RNA in the Xi. SAF has a nuclear transport domain that must be methylated for its nuclear localization [74]. Another protein requiring methylation for nuclear targeting is peptidylarginine deiminase V (PAD-V), an enzyme that converts unmodified arginine residues in polypeptides to citrulline, primarily in histones H2A, H3, and H4 in the nucleus [75]. In the nucleus, PAD-V acts on those few histones that have not received correct post-translational modifications (ex. methylation of arginines). If PAD-V remains in the cytoplasm due to lack of methylation, it could potentially deiminate nascent proteins, such as histones, creating autoantigenic polypeptides. Citrullinated proteins do appear to play a part in autoimmune diseases, such as citrullinated collagen in rheumatoid arthritis [76].

Epstein–Barr virus (EBV) is among the suspected contributing factors in autoimmune diseases. Elevated polyamines are found in EBV-infected lymphocytes [77]. Many viruses, such as EBV, on reactivation will induce expression of the host cell's MYC protein as an initial step [78]. MYC then induces ODC, generating putrescine. Putrescine stimulates increased SAMDC activity and is a precursor in synthesis of free spermidine. This could feed into the hypothesized over-expressed SMS and SAT1. The eventual result would be disruption of methylation in its functions: gene silencing, intracellular signaling, and intracellular protein trafficking.

Concluding remarks

The heterogeneity of the X chromosome, the complexity of the X-inactivation process, and the unique temporal and spatial treatment of the inactivated X chromosome, creates vulnerability in cells. The disruption of XCI and subsequent loss of dosage compensation can originate from genetic aberrations that lead to altered epigenetic control. When the normal epigenetic control of X inactivation is disrupted, even in only a few cells, the potential arises for an autoimmune reaction. Aberrations in methylation appear to be an important aspect in this disruption and possible autoimmune reactions, since methylation impacts signaling, protein trafficking, and gene silencing. Finally as an Editor's note, in addition to this special issue on epigenetics, there have been several recent publications which have focused not only on epigenetics and autoimmunity, but epigenetics as a developmental origin of a variety of human diseases [79–89].

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