HHS Public Access

Author manuscript

Adv Exp Med Biol. Author manuscript; available in PMC 2016 June 17.

Published in final edited form as:

Adv Exp Med Biol. 2016 ; 854: 325–331. doi:10.1007/978-3-319-17121-0_43.

The Role of X-Chromosome Inactivation in Retinal Development and Disease

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Abstract

The expression of X-linked genes is equalized between males and females in mammalian species through X-Chromosome inactivation (XCI). Every cell in a female mammalian embryo randomly chooses one X Chromosome for epigenetic silencing at the 8–16 cell stage, resulting in a Gaussian distribution of XCI ratios with a peak at 50:50. At the tail extremes of this distribution, X-linked recessive mutations can manifest in disease in female carriers if the mutant allele is disproportionately active. The role of XCI skewing, if any, in X-linked retinal disease is still unknown, although many have speculated that such skewing accounts for phenotypic variation in female carriers of X-linked retinitis pigmentosa (XlRP). Some investigators have used clinical findings such as tapetal-like reflex, pigmentary changes, and multifocal ERG parameters to approximate XCI patches in the retina. These studies are limited by small cohorts and the relative inaccessibility of retinal tissue for genetic and epigenetic analysis. Although blood has been used as a proxy for other tissues in determining XCI ratios, blood XCI skews with age out of proportion to other tissues and may not accurately reflect retinal XCI ratios. Future investigations in determining retinal XCI ratios and the contribution of XCI to phenotype could potentially impact prognosis for female carriers of X-linked retinal disease.

Keywords

X-Chromosome inactivation; Dosage compensation; Skewed inactivation; Escape genes; Retinal dystrophies; X-linked retinitis pigmentosa; X-linked retinoschisis; Choroideremia

43.1 Introduction

X-Chromosome Inactivation (XCI) is a dosage compensation mechanism used in mammals to equilibrate the expression of X-linked genes across genders (Lyon 2002). Every cell in the female embryo inactivates either the maternal or the paternal X chromosome, and the inactivation choice is passed down to subsequent daughter cells. This choice is typically

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made at random, although there are exceptions, and the XCI ratio in newborn females follows a normal distribution with a peak at 50:50. Inactivation of the X chromosome is facilitated by expression of *XIST* RNA, which binds to the chromosome of choice and mediates downstream methylation and inactivation (Brown et al. 1991).

XCI is determined at the 8–16 cell stage. This was demonstrated in human embryo studies that showed accumulation of *XIST* RNA starting at the 8-cell stage (van den Berg et al. 2009). Another study modeled distribution curves for XCI ratios based on theoretical numbers of stem cells present at the time of XCI choice. The predictions for 8- and 16-cell embryos most closely fit the empirically determined distribution curve, suggesting that XCI occurs within this window (Amos-Landgraf et al. 2006).

43.2 Escape Genes and Retinal Disease

A subgroup of X-linked genes escapes inactivation and is expressed from both X chromosomes. In a comprehensive study looking at inactivation status of 612 X-linked genes in human-rodent hybrid cells, 15 % of genes escaped inactivation, and an additional 10 % showed variable inactivation between individuals (Carrel and Willard 2005). Escape genes were often expressed at lower levels from the inactivated chromosome compared to the active chromosome. Both Retinitis Pigmentosa GTPase Regulator (*RPGR*) and *RP2*, which are together responsible for > 90 % of X-linked retinitis pigmentosa (XLRP), were found to be completely silenced. See Table 43.1 for a complete list of X-linked genes associated with retinal disease and their inactivation status in the hybrid cell lines (Carrel and Willard 2005; Daiger 2014).

43.3 XCI Skewing

Skewing of the XCI ratio from the expected 50:50 ratio can occur at the time of XCI choice in the early embryo (primary), or during embryonic development or later in life (secondary). In mice, XCI choice is greatly biased by variation at the X Controlling Element locus (*XCE*) on the X chromosome (Courtier et al. 1995; Chadwick and Willard 2005). In humans, nonrandom XCI choice occurs due to mutations in X-linked genes, including the *XIST* gene (Plenge et al. 1997).

Disease-causing X-linked mutations often bias cell survival and replication during development and cause secondary XCI skewing (Orstavik 2009). For example, in Lesch-Nyhan Syndrome and Menkes disease, cells with a normal active X chromosome have a growth advantage over cells with a mutant active X (Migeon 2007; Desai et al. 2011). In contrast, some female carriers of Duchenne Muscular Dystrophy and Hemophilia A demonstrate preferential inactivation of the wild-type allele and can manifest disease (Pegoraro et al. 1994; Di Michele et al. 2014). This pattern appears to be heritable in some cases (Renault et al. 2007; Esquilin et al. 2012), indicating that either the disease locus or another genetic modifier is biasing XCI in these families.

Even in the absence of a pathologic mutation XCI ratios skew with age, in some tissues more than others (Hatakeyama et al. 2004; Amos-Landgraf et al. 2006). Blood is particularly prone to XCI skewing with time, and blood has shown increased XCI skewing compared to

buccal mucosa, skin, muscle, and urinary epithelium (Sharp et al. 2000; Knudsen et al. 2007; Bolduc et al. 2008). Only 4.9 % of newborns show skewing > 80:20 in blood compared to 14.2 % of adults (Amos-Landgraf et al. 2006). This is particularly relevant because blood is the most frequently sampled tissue in the literature for determining XCI ratios and may not always be a good proxy for the tissue of interest. For example, in severely affected female carriers of X-linked ornithine transcarbamylase deficiency, skewed XCI was found in the liver, but not in the blood (Yorifuji et al. 1998).

There is very little data on correlation of XCI in the retina compared to blood. In one study that examined multiple tissues at autopsy from a female affected with Leber's Hereditary Optic Neuropathy, the XCI ratio in retina was 43:57, compared to 65:35 in blood and 56:44 in optic nerve (Pegoraro et al. 2003). Not only was the ratio more skewed in blood than in retina, but it was also skewed in the opposite direction.

43.4 XCI Patches in the Retina

Due to the relative inaccessibility of human retina tissue for investigation, XCI patches in the retina have largely been studied in animal models. The mouse retina displays clonal patches of XCI in a radial pattern. XCI occurs between E5.5 and E8.5 in mice, and at day E10.5 female mice heterozygous for an X-linked *lacZ* transgene showed random intermingling of *lacZ* active and inactive cells, indicating free migration of neuroepithelial cells. At birth, the mouse retinas showed alternating columns of *lacZ* active and inactive cells, indicating that the progenitor cells became fixed in location at some point (Reese and Tan 1998; Smallwood et al. 2003). Cone, horizontal, amacrine, and ganglion cells were interspersed into non-matching columns, suggesting tangential migration of these cells (Reese and Galli-Resta 2002).

In XLPRA2, a canine model of XLRP, carrier female dogs displayed patches of mislocalization of rod opsin at 3.9 weeks, followed by outer segment disruption and rod loss in these patches, which the authors attributed to patches of inactivation of the wild-type allele (Beltran et al. 2009). Older dogs by 39 weeks of age had a more uniform, although thinner, outer nuclear layer, which the authors speculated may result from early migration of healthy rods into diseased areas.

Adaptive optics was used to examine the cone mosaic in a human female carrier of protan color-blindness (deficiency of L-opsin on one X-chromosome) (Hofer et al. 2005). If cones were organized into XCI patches, one would see patches of M-cones devoid of L-cones. Instead, the L, M, and S cones were randomly dispersed in the fovea. The ratio of L:M cones was 0.37:1 (or 27 % L cones), suggesting an XCI ratio of approximately 54:46. This interspersion of cones is consistent with prior studies demonstrating migration of cones into the fovea during fetal development (Yuodelis and Hendrickson 1986; Diaz-Araya and Provis 1992). It is unknown whether rods are distributed in XCI patches in the adult human retina.

43.5 XCI Patches and Skewing in Retinal Disease

XCI has been investigated in several X-linked retinal diseases, including XLRP, choroideremia, and retinoschisis. XLRP in particular is known for variable manifestation in

female carriers, and differences in XCI ratios have been proposed as a chief mechanism for this variation. To date, investigations have been performed in small groups of patients using blood to determine XCI ratios. In one study involving three families with the same *RPGR* mutation, XCI ratios in blood were not associated with carrier phenotype (Banin et al. 2007). Of note, two families had unaffected carriers and shared a common haplotype, while the third family had severely affected carriers with a different surrounding haplotype, suggesting a linked genetic modifier affecting phenotype. Others have reported patchy disease in female carriers of XLRP (Szamier and Berson 1985; Cideciyan and Jacobson 1994; Banin et al. 2007). In one study of multifocal electroretinography (mfERG) in five clinically unaffected female carriers of XLRP, two carriers demonstrated patches of reduced amplitude, and three carriers demonstrated patches of implicit time delay. However, these patches did not correlate with each other and did not correlate with patches of tapetal-like reflex (Vajaranant et al. 2002).

In a study of seven obligate carriers of X-linked choroideremia, one carrier showed visual field abnormalities, six carriers showed patches of significant implicit time delays on mfERG, and four of these six also showed overlapping patches of significantly reduced amplitude (Vajaranant et al. 2008). All carriers had patches of pigmentary retina changes on fundoscopic exam, although these patches did not always correlate with areas of reduced function on mfERG. In two families with X-linked choroideremia, no link was found between female carrier phenotype and XCI skewing in peripheral blood (Perez-Cano et al. 2009).

Carriers of X-linked retinoschisis (XLRS) are generally not affected. There are rare reports of fundoscopic and psychophysical abnormalities (Ali et al. 2003; Rodriguez et al. 2005). In a study of mfERG in nine obligate carriers of XLRS, two carriers showed patches of significant implicit time delay that overlapped almost perfectly with patches of significantly reduced amplitude (Kim et al. 2007).

43.6 Conclusion

The studies described above have yielded variable results, and the extent to which XCI ratios contribute to X-linked retinal diseases remains controversial. Of note, these studies have all included very small numbers of patients, and those that looked at XCI ratios did so in blood samples. Given the notoriety of blood for instability of XCI populations and increased XCI skewing with age, this tissue may be a particularly poor proxy for retina tissue despite the advantage of accessibility. Future studies would benefit from larger cohorts and exploration of XCI ratios in other accessible tissues with potentially more stable XCI. Determining the contribution of XCI ratios to phenotype could have prognostic utility for carriers of X-linked retinal diseases. In particular, female carriers of XLRP vary in phenotype from unaffected to severely affected and may benefit from prognostic information, which is currently lacking. In addition, with gene therapy on the horizon for XLRP, prognostic factors may play an important role in selecting appropriate female candidates for intervention.

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Table 43.1

Retinal disease genes and inactivation status. (Carrel and Willard 2005; Daiger 2014)

Gene or locus (alias)	Disease	Inactivation
OFD1 (RP23, CXORF5)	Joubert syndrome, orofaciodigital syndrome 1, Simpson-Golabi, Behmel syndrome 2, retinitis pigmentosa	Escapes inactivation
RS1 (XLRS1)	Retinoschisis	Variable escape
RP6	Retinitis pigmentosa	Not determined
DMD	Oregon eye disease	Variable escape
OPA2	Optic atrophy	Not determined
NYX (CSNB1, CSNB1A, CSNB4)	Congenital stationary night blindness	Not determined
COD1	Cone dystrophy	Not determined
RPGR (CORDX1, RP3)	Retinitis pigmentosa, cone dystrophy	Inactivated
PRD	Primary retinal dysplasia	Not determined
NDP (EVR2)	Norrie disease, familial exudative vitreoretinopathy, Coats disease	Not determined
AIED (OA2)	Åland island eye disease	Not determined
CACNA1F (CORDX3, CSNB2, CSNB2A, CSNBX2)	Congenital stationary night blindness, ÅIED-like disease, cone-rod dystrophy	Inactivated
RP2	Retinitis pigmentosa	Inactivated
PGK1	Retinitis pigmentosa with myopathy	Inactivated
CHM	Choroideremia	Variable escape
TIMM8A (DDP, DDP2, DFN1)	Optic atrophy with deafness-dystonia syndrome	Variable escape
RP24	Retinitis pigmentosa	Not determined
COD2 (CORDX2)	Cone dystrophy	Not determined
RP34	Retinitis pigmentosa	Not determined
OPN1LW (BCM, CBP, COD5, RCP)	Deuteranopia, macular dystrophy in blue cone monochromacy with loss of locus control element	Not determined
OPN1MW (CBD, GCP)	Protanopia, macular dystrophy in blue cone monochromacy with loss of locus control element	Not determined

The table includes a comprehensive list of X-linked genes and loci known to be associated with retinal phenotypes and their inactivation status on the inactivated X chromosome in human-rodent hybrid cell lines