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Tumor suppressor genes that escape from X-inactivation contribute to cancer sex bias

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Abstract

There is a striking and unexplained male predominance across many cancer types. A subset of X chromosome (chrX) genes can escape X-inactivation, which would protect females from complete functional loss by a single mutation. To identify putative “Escape from X-Inactivation Tumor Suppressor” (EXITS) genes, we compared somatic alterations from >4100 cancers across 21 tumor types for sex bias. Six of 783 non-pseudoautosomal region (PAR) chrX genes (*ATRX, CNKSR2, DDX3X, KDM5C, KDM6A*, and *MAGEC3*) more frequently harbored loss-of-function mutations in males (based on false discovery rate <0.1), compared to zero of 18,055 autosomal and PAR genes (P<0.0001). Male-biased mutations in genes that escape X-inactivation were observed in combined analysis across many cancers and in several individual tumor types, suggesting a generalized phenomenon. We conclude that biallelic expression of EXITS genes in females explains a portion of the reduced cancer incidence compared to males across a variety of tumor types.

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A.D., V.S., S.E.S., J.P.C., A.Y., T.J.S., and J.M.H designed, performed, and analyzed computational and laboratory analyses. D.M.W., A.A.G, R.B., M.S.L, G.G., and A.A.L. conceived the study, designed experiments, and interpreted the data. A.D., D.M.W., M.S.L, G.G., and A.A.L. wrote the paper.

Competing Financial Interests Statement

The authors have no relevant competing financial interests to disclose.

Introduction

Based on SEER (Surveillance, Epidemiology, and End Results Program) data from 2008–2012, US males carry an age-adjusted excess risk of 20.4% for developing any cancer (516.6 versus 411.2 for females per 100,000 person-years) and ≥2:1 male predominance for some individual cancer types[1]. This excess risk results in approximately 153,000 additional new cases of cancer in US men annually. Yet, the male predominance in cancer incidence remains largely unexplained[2]. The disparities between men and women occur across the world, even after adjusting for differences in gross domestic product, geographical region, and environmental risk factors including tobacco exposure[1, 3]. In fact, changes in tobacco use among males and females over the past two decades have resulted in a marked reduction in the male:female (M:F) ratio of lung and bronchus cancer (Figure 1a). However, over the same time period, the M:F ratios for several cancers have remained >2:1, including those associated with tobacco use such as kidney and renal pelvis, urinary bladder, and oral cavity and pharynx cancers (Figure 1a, Supplementary Figure 1).

Previous reports have identified chrX genes outside of the pseudoautosomal region (PAR) with higher mutation frequencies in male cancers compared to female cancers[4–6]. For example, *KDM6A* at Xp11.2, which encodes the histone lysine demethylase UTX, has loss-of-function mutations more predominately in male cancers across multiple subtypes[4, 7]. Among female cancers with *KDM6A* mutations, homozygous mutations are common[7], suggesting that some tumor suppressor genes (TSGs) on chrX outside of the PAR follow the classic Knudson two-hit hypothesis in females[8]. This poses a conundrum because one chrX in all female cells undergoes X-inactivation during embryogenesis, which should leave each female cell functionally haploid for non-PAR genes on chrX[9]. However, a fraction of chrX genes “escape” inactivation and have biallelic expression, albeit with poorly understood mechanisms leading to differences across individuals and cell types[10–14].

We hypothesized that mutations in TSGs that escape X-inactivation could underlie a significant fraction of excess male cancers, as males would require only a single deleterious mutation while females would require two (Figure 1b-c). We termed these genes ‘EXITS’, for *E*scape from *X*-*I*nactivation *T*umor *S*uppressors (EXITS). In females with a mutation in an EXITS gene, a corollary of the EXITS hypothesis is that the other allele will be mutated or deleted (Figure 1c). ChrX is among the most frequently aneuploid chromosomes in female cancers[15], and alteration of X-inactivation by targeted deletion of *Xist* promotes tumorigenesis[16].

Similarly, Y chromosome (chrY) loss is observed in ≥30% of male renal cell, head and neck, and bladder cancers[15, 17, 18]. Age-related loss of chrY in non-malignant blood cells is frequent, is increased among tobacco users, and is associated with an approximately 3.5-fold higher risk for developing a non-hematological cancer[19, 20]. The Y chromosome harbors evolutionarily ancestral homologs of a small fraction of non-PAR chrX genes. ChrX genes with chrY homologs are known to be more likely to escape X-inactivation[21, 22]. In some cases, chrY genes can rescue viability or other phenotypes upon loss of their chrX homologs[23, 24], although for most genes there is not clear evidence of functional redundancy. Thus, a second corollary to the EXITS hypothesis is that chrY loss will co-

occur in male tumors that have mutated an EXITS gene with a functional chrY homolog (Figure 1c).

Results

Male-biased loss-of-function mutations on the X chromosome in cancer

To test the EXITS hypothesis, we performed an unbiased analysis of paired tumor/germline exome sequencing data from 4126 patients across 21 tumor types from The Cancer Genome Atlas (TCGA) and Broad Institute datasets (Supplementary Table 1, Supplementary Figure 2). In 1994 cases, copy number variation data was also available based on high-density single nucleotide polymorphism (SNP) arrays. The tumors analyzed excluded sex-restricted cancers such as prostate and ovarian cancer, as well as breast cancer.

We used a conservative mutation classification algorithm to ensure that the included variants were restricted to somatic truncating and missense alterations that were most likely loss-of-function ("LOF", see Methods), or DNA copy number (CN) loss of at least one allele (Supplementary Figure 3). We applied a permutation analysis to determine which genes were mutated in males at frequencies higher than expected based on the overall mutation rates in males and females for each tumor type and normalized to the number of X chromosomes. We performed this analysis for only LOF mutations (SNVs and InDels) in the 4126 patients with exome data and then again for LOF mutation or CN loss (LOF mutation/CN loss) in the 1994 patients with both exome and copy number data available.

At a false discovery rate (FDR) <0.1, there were no autosomal or PAR genes (n=18,055) that had LOF mutations more frequently in male cancers. In addition, at FDR<0.1 no genes on chrX had a significantly increased frequency of silent coding mutations in male cancers, and no genes on chrX had non-silent LOF mutations more frequently in female cancers (Supplementary Tables 2–4). In contrast, at FDR<0.1, six of 783 non-PAR chrX genes (*ATRX, CNKSR2, DDX3X, KDM5C, KDM6A*, and *MAGEC3*) harbored LOF mutation or LOF mutation/CN loss more frequently in male cancers (P<0.0001 compared to zero of 18,055 autosomal or PAR genes; Table 1, Figure 2a-b, and Supplementary Tables 5–6). Of note, *ATRX*[25, 26], *DDX3X*[27], *KDM5C*[28], and *KDM6A*[29] have been previously implicated as TSGs, via recurrent loss-of-function alterations in cancer genomes and/or by direct experimental evidence.

We performed the same permutation analysis in each of the 21 cancer types individually to discover tissue- or disease-restricted EXITS genes. LOF mutation/CN loss was enriched in *ATRX* among male lower-grade gliomas (LGG; FDR<10^{-4}) and in *KDM5C* among male clear cell kidney cancers (KIRC, FDR=0.044) (Table 1, Figure 2c-d). There were no autosomal or PAR genes with male-biased LOF mutation in any of the individual cancer types at FDR<0.1.

To assess the robustness of these findings, we then used a second statistical test on the same dataset based on a log likelihood ratio, which also normalizes to the background male and female mutation rates in each tumor type and the number of X chromosomes (see Methods). Five (*CNKSR2, DDX3X, KDM5C, KDM6A*, and *MAGEC3)* of the 6 genes were re-

discovered as significantly more frequently mutated across all male cancers in this analysis (FDR<0.1), as were *ATRX* in male LGG and *KDM5C* in male KIRC (Supplementary Figure 4 and Supplementary Table 7).

EXITS gene alterations associated with excess male cancers

Adult lower-grade gliomas occur at a M:F ratio of ~1.4:1 (or 58.3% male:41.7% female)[30]. Considering that 42% of male LGGs in our dataset had *ATRX* mutations compared to 26% of female LGGs, approximately 80% of the excess male LGGs in our cohort had *ATRX* mutations. Similarly, 8.6% of the excess male head and neck squamous cell carcinomas had *KDM6A* mutations and 12.1% of the excess male clear cell kidney cancer had *KDM5C* mutations (see Methods for calculations). However, these figures are undoubtedly an underestimate of the total contribution of male-biased alterations in these diseases because they only consider stringently defined, loss-of-function mutations within coding regions, and they are limited by the sample size of our datasets.

To address the latter limitation, we calculated the number of tumor/normal pairs needed for 80% power to detect a coding LOF mutation on chrX with a significant male bias given different M:F disease incidence ratios, basal mutation rates, and prevalence of an alteration in a specific population (Figure 3a). For example, clear cell kidney cancer (KIRC) has a M:F incidence ratio of ~2:1 and therefore, as expected, ~50% of all mutations on the X chromosome across all KIRCs are in males because they have only one chrX. If an EXITS gene were mutated in 5% of all KIRCs it would require sequencing >1000 tumors to have 80% power to detect a 4-fold male mutation bias. Therefore, many additional cancers, including hundreds of tumor types not represented in this dataset, will need to be assessed before the contribution of EXITS genes to overall excess male cancer risk can be fully quantified. This calculation provides target sample sizes for such studies.

The expression of EXITS genes could also be downregulated in the absence of coding mutations by several mechanisms, including non-coding mutations or epigenetic changes. We analysed exome and RNA sequencing (RNA-seq) data by patient sex from head and neck squamous cancer and clear cell kidney cancer. Essentially all tumors with downregulation of EXITS genes (defined as expression <5th percentile of the male cancers) in the absence of a DNA mutation were from males (22/469 males vs 2/218 females for *DDX3X*, P=0.012 by Fisher's exact test; 16/450 males vs 2/216 females for *KDM5C*, P=0.071; 23/465 males vs 2/217 females for *KDM6A*, P=0.0077; Figure 3b). Whole genome sequencing of a large number of tumors will be necessary to determine whether noncoding mutations on chrX underlie EXITS gene downregulation.

EXITS gene mutations associated with loss of the paired chrY or chrX

Next, we asked whether additional genes that escape X inactivation or have Y homologs may also function as EXITS genes. We compiled a list of 59 chrX genes with evidence of X-inactivation escape in multiple contexts from studies of human lymphoblastoid cells and hybrid fibroblasts[12, 13, 31] (Supplementary Table 8). These 59 genes had a higher M:F ratio of LOF mutation/CN loss compared to other chrX genes (P=0.022; Supplementary Figure 5a). Similarly, we identified 17 chrX genes with predicted functional Y homologs[21]

(Supplementary Table 8) and found that they had a trend toward higher M:F ratio for LOF mutation/CN loss compared to other chrX genes (P=0.058; Supplementary Figure 5b).

If we exclude the 6 genes we identified as EXITS genes, there was no discernible male mutation bias among either the 56 remaining escape genes or the remaining 14 chrX genes with functional chrY homologs (Figure 3c-d). However, we may have failed to identify additional EXITS genes from our dataset because of power limitations that result from agnostically assessing all coding genes. A permutation test limited to genes previously reported to either escape X-inactivation (n=59) or have chrY homologs in humans or in recent mammalian evolution (n=33)[21] identified two additional genes with higher frequencies of LOF mutation and/or CN loss in male cancers (FDR<0.1): *NLGN4X* and *RBM10* (Supplementary Table 9). Previous functional data supported *RBM10* as a *bona fide* tumor suppressor gene[32].

We next tested the hypothesis that EXITS genes will harbor biallelic inactivation when mutated in female tumors. We used SNP array data to infer which female tumors had lost an entire chrX and compared this to the incidence of LOF mutation or focal CN loss on the remaining chrX (Supplementary Figure 3). Female tumors with EXITS mutations were more likely to have lost the whole other chrX than tumors without EXITS mutations (23.3% [10/43] vs 6.2% [45/726], P=0.0005 by Fisher's exact test), suggesting an enrichment for biallelic loss. In addition, 6.3% of the female tumors with an EXITS mutation had two LOF mutations in that gene, although with short read sequencing we are unable to determine whether these are in *cis* or *trans*.

To determine whether chrY loss is enriched among male tumors with EXITS gene mutations, we utilized a conservative approach to classify chrY copy number based on exome sequencing data across 1443 male tumors in our dataset (Supplementary Figure 6 and Supplementary Methods). Male tumors with loss-of-function mutations in any of the three EXITS genes with Y homologs (*DDX3X, KDM5C*, and *KDM6A*) had a trend toward enrichment for chrY loss compared to tumors without mutations (10.2% [9/88] versus 5.8% [78/1355], P=0.077). Male tumors with an LOF mutation in any chrX gene with a predicted functional Y homolog were more likely to have lost chrY than those without (10.6% [15/142] versus 5.5% [72/1301], P=0.019). Therefore, age- and tobacco-associated spontaneous loss of chrY could disproportionately increase the frequency of some cancers in males, as LOF mutation/CN loss of EXITS genes with functional chrY homologs would lead to complete gene inactivation in male cells that have lost chrY (Figure 1c)[19].

To assess the relative functional contribution of X-X pairs in females compared to X-Y homologs in males, we determined the rate of concurrent mutation and chrX loss in female tumors with the rate of concurrent mutation and chrY loss in male tumors for the three EXITS genes with Y homologs (*DDX3X, KDM5C*, and *KDM6A*). Female tumors with LOF/CN mutations were more likely to lose chrX than male tumors with LOF/CN mutations were to lose chrY (36% [9/25] versus 8.2% [6/73], P=0.0022). This result suggests that the chrY homologs in males may not have equivalent tumor suppressor activity to EXITS gene alleles on the inactivated chrX in females.

ATRX and CNKSR2: hypothesis generation from sex-biased mutation patterns

Among the EXITS genes we identified, *DDX3X, KDM5C*, and *KDM6A* are recognized escape genes across multiple tissues[12, 13]. *CNKSR2* and *MAGEC3* were more recently suggested to escape as determined by next-generation sequencing and epigenetic analyses[33]. There are data in human cell lines showing *ATRX* escape using RNA-FISH[34, 35] but *ATRX* has not been traditionally classified among the escape genes[12, 13, 21, 31]. The *ATRX* locus is located in a chrX region that contains multiple escape genes and/or Y homologs in lower mammals[21] and *ATRX* escapes in human trophoblast cells[36]. Nearly all of the excess male cancers with *ATRX* mutations in our dataset were in lower-grade gliomas (Table 1). We therefore hypothesized that *ATRX* may escape X-inactivation, but only in certain contexts possibly including in the brain. Tissue-specific and inter-individual variation in escape are recognized phenomena that have been described previously[14, 33].

We took two approaches using RNA-seq to demonstrate evidence of X-inactivation escape in putative EXITS genes in tumors and normal tissues: allele-specific expression in female cells and male vs. female expression levels. At germline heterozygous single nucleotide polymorphism (SNP) sites in coding regions from tumors without somatic mutations in the tested genes, we observed evidence of biallelic expression of *ATRX, KDM6A, KDM5C*, and *DDX3X* in one or more of six tumor types (GBM, LGG, HNSC, KIRC, LUAD, LUSC) in which we had identified male-biased mutations (Supplementary Figure 7). This included tumor types where male-biased mutations were significant in single cancer analysis (e.g., *ATRX* in LGG, *KDM5C* in KIRC). We also analyzed female and male expression levels of EXITS genes in tumors without mutations, because escape from X-inactivation often results in higher expression in female cells[11]. *DDX3X, KDM5C*, and *KDM6A* had higher expression in non-mutated female compared to male tumors across all types tested. Notably, *ATRX* was only higher in female LGG and *CNKSR2* only in female LUAD, the tumor types for each gene where the male loss-of-function mutation bias was also seen (Supplementary Figure 8).

To analyze biallelic expression in normal tissues, we queried data from the Genotype-Tissue Expression (GTEx) Project, which includes RNA-seq from multiple tissue types in non-diseased individuals[37]. Within local tissue environments X-inactivation can be skewed toward one chromosome as a result of a shared developmental origin[38], which might allow detection of escape from X-inactivation if the GTEx biopsy demonstrated higher minor allele expression for escape compared to ‘known’ non-escape genes. Indeed, that is what we observed for EXITS genes, including high minor allele expression of *ATRX* in brain biopsies from several donors in multiple anatomic sites suggesting X-inactivation escape ($P<0.0001$ vs non-escape genes in cortex, $P=0.0224$ in cerebellum by K-S test; Supplementary Figure 9).

We also analyzed EXITS gene expression by sex in normal tissue biopsies from GTEx. In contrast with other tissues or in male brain, *ATRX* expression in female brain showed two distribution peaks and was best fit by models of bimodality ($P=0.04$; Supplementary Figure 10a-b). We compared female and male expression for the six EXITS genes across multiple tissues and found that while most have higher expression in females in all measurable sites, evidence for *ATRX* escape is limited to female brain (Supplementary Figure 10c). These

data also suggest that *ATRX* undergoes heterogeneous escape from X-inactivation in female brain. Further studies will be required to understand if *ATRX* escape is restricted to certain brain cell subsets or regions, or if there is inter-female heterogeneity in escape status, and how these could contribute to male-bias among *ATRX*-mutated gliomas.

CNKSR2 was not previously implicated as a tumor suppressor gene in large sequencing studies[39]. It encodes a putative Ras pathway scaffolding protein implicated in an X-linked neurodevelopmental disorder[40]. Depletion of *Cnksr2* from murine 3T3 fibroblasts using two independent lentiviral shRNAs resulted in expression changes that were enriched in genes associated with oncogene signatures by Gene Set Enrichment Analysis (GSEA) in the Molecular Signatures Database (MSigDB, Broad Institute) C2:CGP collection[41], including transformation by HRAS, KRAS, and the Ras-like protein RHOA (Supplementary Figure 11a-c, Supplementary Table 10). In addition, we queried the 50 “Hallmarks” MSigDB datasets of general cellular function[42], a combined automatic and manual curation of >10,000 gene sets to overcome problems of redundancy and heterogeneity in GSEA. *Cnksr2* depletion was most enriched with signatures of MTOR and KRAS signaling (Supplementary Figure 11d, Supplementary Table 11). Consistent with activation of the RAS/MAPK pathway, cells expressing *Cnksr2* shRNAs had increased ERK phosphorylation and enhanced colony-forming activity in soft agar, consistent with *in vitro* transformation (Supplementary Figure 11e-g). These findings demonstrate how genetic-epidemiologic associations such as sex bias may help distinguish driver from passenger events, and possibly identify cancer-associated genes with a higher likelihood of functional relevance.

Discussion

Escape from X-inactivation results in expression of two copies of a tumor suppressor gene in females whereas males only have one. The EXITS hypothesis is that biallelic expression of these genes affords females with enhanced cancer protection, which substantively contributes to the observed higher incidence of some tumors in males. Our data have provided evidence to support this hypothesis, as the genes with an increased incidence of loss-of-function mutations in males across many cancer types were exclusively non-PAR genes on chrX, including several that are known to be TSGs and/or known to escape X-inactivation in certain contexts. Genes that do not normally escape X-inactivation could also function as EXITS in some situations, as it is recognized that aberrant or ‘leaky’ X-inactivation/escape can occur in cancer cells[35, 43]. Additional studies are needed to identify the full complement of sex-biased cancer genes; these include the sequencing of more tumor/normal pairs to increase statistical power, assessments of non-coding genomes for additional mechanisms of EXITS gene inactivation, and the interrogation of additional tumor types not represented herein to identify disease-restricted sex-biased TSGs.

Undoubtedly, EXITS gene mutations are not the sole explanation for differences in cancer incidence between men and women. Alcohol use, tobacco exposure, and endocrine biology are known to affect cancer epidemiology, with the latter possibly causing female predominance of some cancers. However, these environmental and hormonal factors associated with sex-specific differences in cancer could interact with EXITS loci or their gene products to modulate cancer risk. For example, a clastogenic carcinogen could

disproportionately affect men if the process of carcinogenesis involves inactivation of an EXITS gene or loss of chrY, as has been shown for tobacco smoking[19]. Similarly, enhancers or other regulatory elements on chrX that modulate autosomal gene transcription *in trans* could qualify as “noncoding EXITS” if their loss promotes carcinogenesis.

Another implication of our data is that male and female tumors of the same cancer type may have distinct genetics, separate from the number of X and Y chromosomes. These differences could be directly related to the impact of EXITS mutations on disease biology or drug response. Alternatively, they could result in more general effects in a population because male and female cancers of a specific type may be effectively distinct diseases (*i.e.*, male tumors without EXITS mutations might behave similarly to female tumors, while EXITS-mutated cancers could have their own biology). To begin to explore these possibilities, we propose that clinical oncology studies should be statistically powered to understand sex-specific differences in outcomes that result from distinct tumor genetics. In addition, pre-clinical models can be generated to address the relative contributions of cell-intrinsic and cell-extrinsic differences between male and female cancers.

Finally, we note that several of the EXITS genes identified herein have also been implicated in germline genetic neurodevelopmental diseases[40, 44–47], and that non-malignant disorders with a significantly higher risk among males (*e.g.* autism[48], schizophrenia[49]) have been associated with polymorphisms on chrX. These data suggest that, besides the obvious link between male sex and monogenic diseases associated with chrX gene mutation, the haploid nature of chrX in males may increase the risk of developing polygenic diseases other than cancer.

Data Availability

RNA-seq data are available through Gene Expression Omnibus (GEO) accession code GSE85462.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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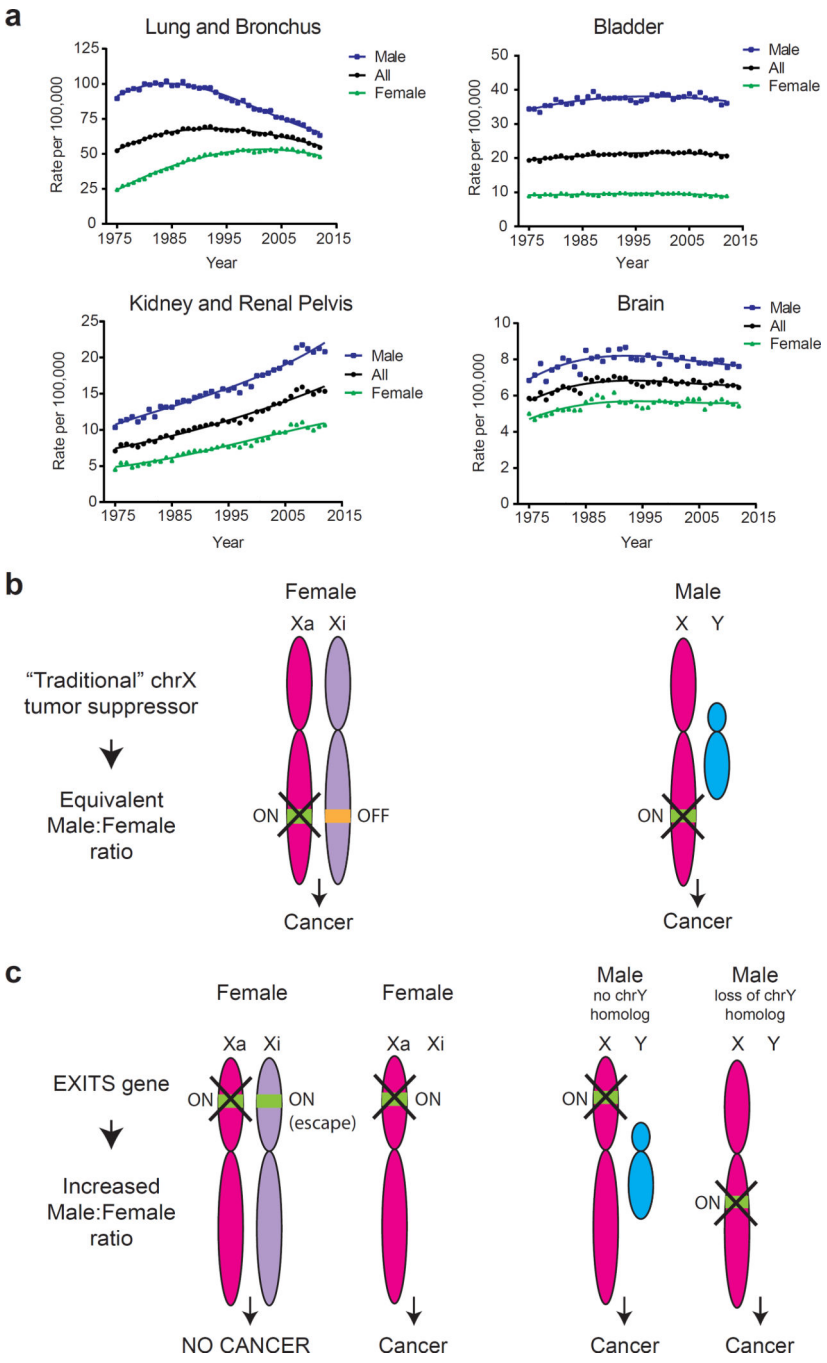

Figure 1. Escape from X-inactivation Tumor Suppressor (EXITS) genes

(**a**) SEER data of annual incidence rates over time for the indicated cancer types in males (blue), females (green), or all patients (black). (**b-c**) The EXITS hypothesis: (**b**) "Traditional" tumor suppressor genes (TSGs) on chrX are represented in the top row for females and males. A single deleterious mutation in a TSG is equally likely to occur in male and female cancers because males have only one chrX, and females have one active chrX (Xa, pink) and one inactive chrX (Xi, purple). (**c**) On the bottom row is a model for EXITS gene behavior. In females, there are two active alleles of EXITS genes and therefore females

are protected from complete gene loss after a single alteration. Complete inactivation of an EXITS gene may require biallelic mutations, or mutation with loss of the other chrX. In males, one mutation could inactivate the only allele of an EXITS gene that has no functional Y homolog, and therefore males would be more likely to develop cancers associated with mutations in those TSGs. Alternatively, because some genes that escape X inactivation have chrY homologs with redundant function, cancers with mutations in those genes would be more likely to occur in males who also have somatic loss of chrY.

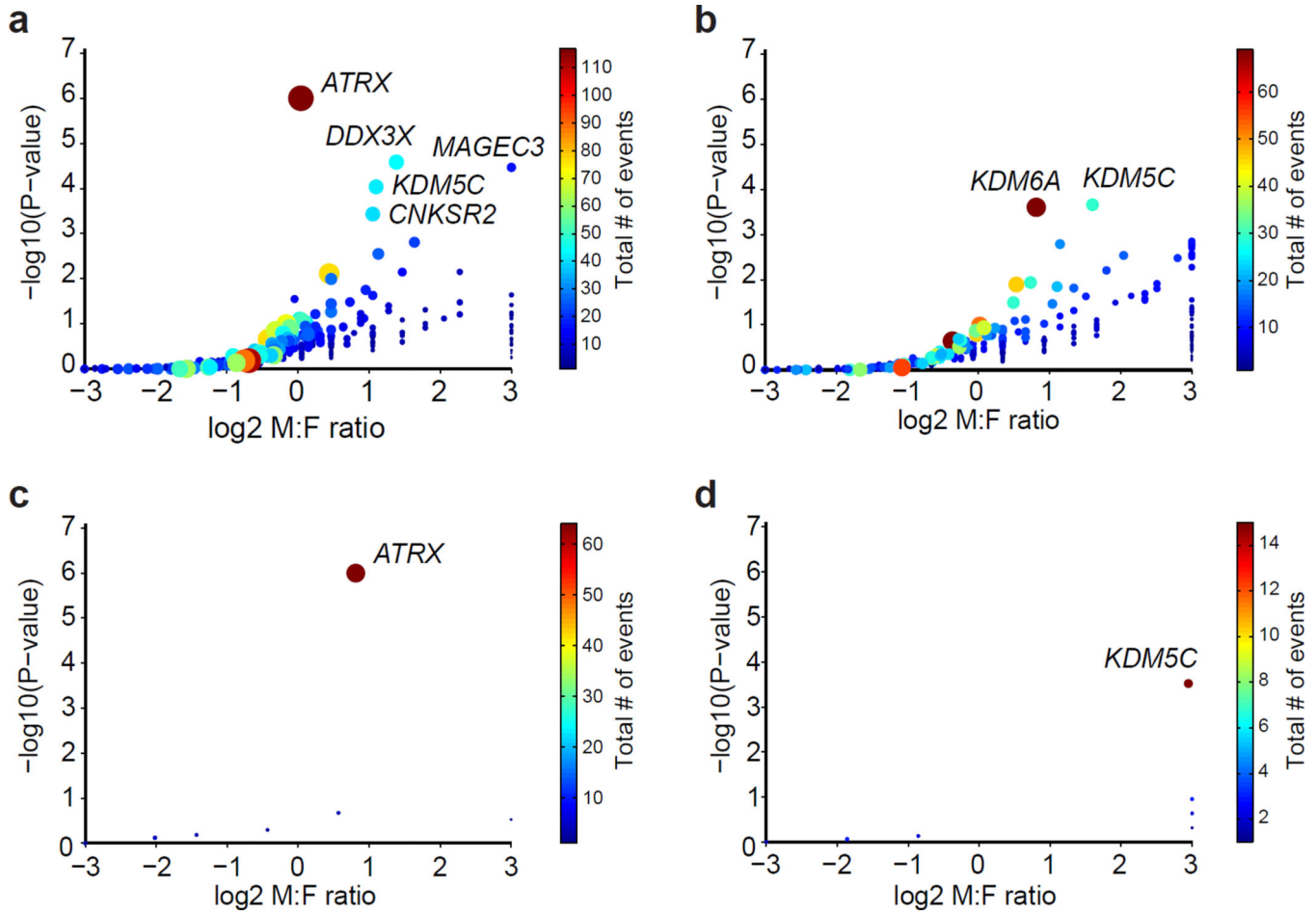

Figure 2. Genes with higher frequencies of somatic loss-of-function (LOF) alterations in male cancers

Permutation testing for genes on chrX across all cancer datasets is shown. The $\log_2$ M:F ratio of events is plotted for each gene against the significance (P) value. The size and color of each circle represent the number of (**a**) LOF mutations, or (**b**) LOF mutation/CN loss events in that gene. Genes with significantly higher (FDR<0.1) frequencies of mutation in male cancers are identified. Disease-specific permutation testing of LOF mutations in (**c**) lower-grade glioma and (**d**) clear cell kidney cancer is shown.

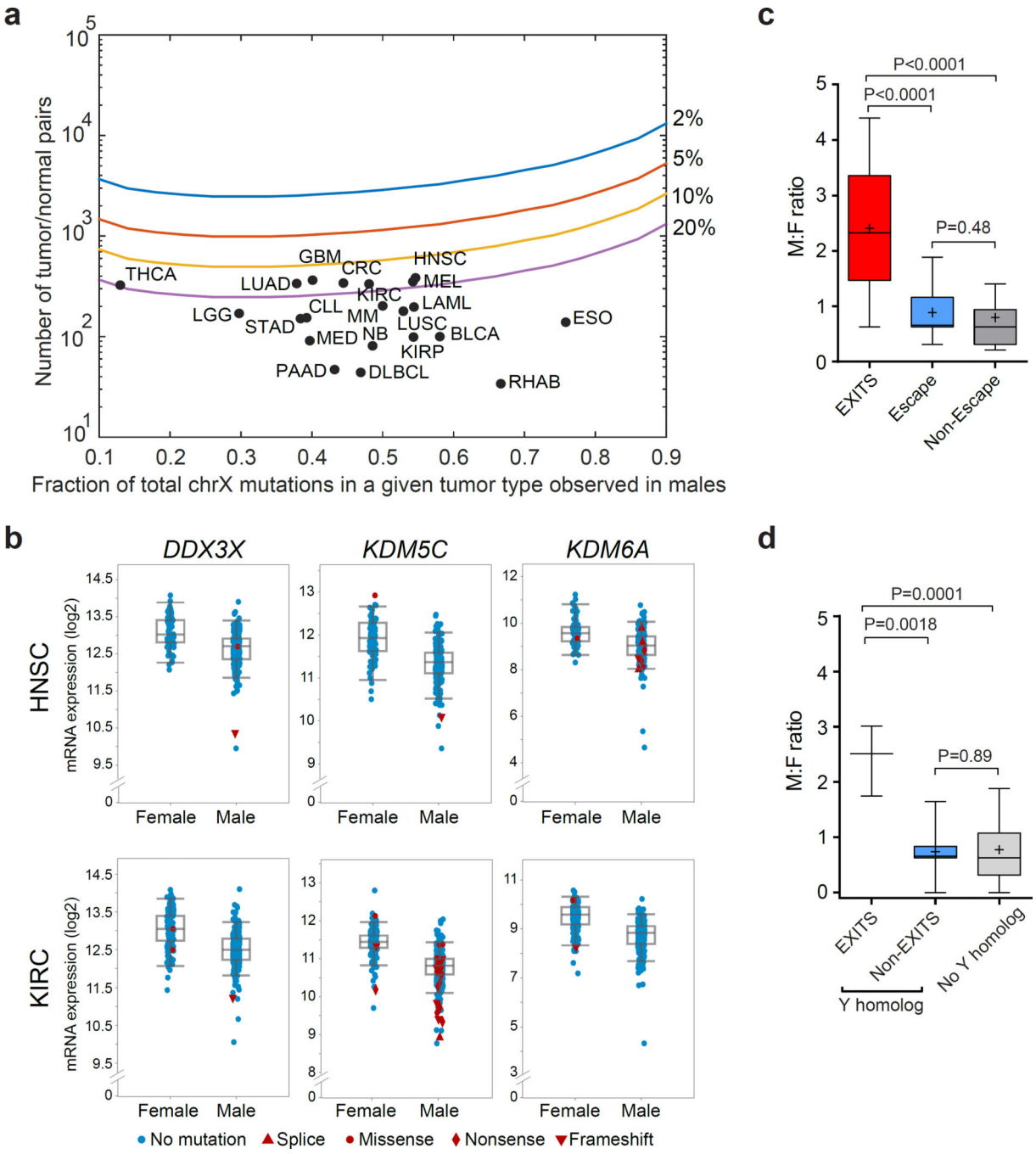

Figure 3. EXITS gene alterations are associated with male cancers

(**a**) Calculation of the number of tumor/normal pairs needed for 80% power to detect 4-fold male-biased LOF mutations with FDR<0.1 (i.e., 4x more prevalent in male than female tumors). The x-axis represents the fraction of all mutations on chrX occurring in males in the cohort (a function of the M:F ratio of disease incidence and overall mutation rate in males and females). Lines represent the percentage of cancers in a given tumor type that harbor a specific mutation (blue, 2%; red, 5%; yellow, 10%; purple, 20%). Each of the 21 tumor types we analyzed is plotted to show the power we had to detect a male-biased

mutation based on the fraction of mutations on chrX in males and number of tumors/normal pairs in the dataset. BLCA, bladder carcinoma; CLL, chronic lymphocytic leukemia; CRC, colorectal carcinoma; DLBCL, diffuse large B cell lymphoma; ESO, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous carcinoma; KIRC, clear cell kidney cancer; KIRP, papillary kidney cancer; LAML, acute myeloid leukemia; LGG, lower-grade glioma; LUAD, lung adenocarcinoma; LUSC, lung squamous carcinoma; MED, medulloblastoma; MEL, melanoma; MM, multiple myeloma; NB, neuroblastoma; PAAD, pancreatic ductal adenocarcinoma; RHAB, rhabdoid tumor; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma. (**b**) RNA-seq expression levels (log_2) for *DDX3X, KDM5C*, and *KDM6A* in head and neck squamous carcinoma (HNSC) and clear cell kidney cancer (KIRC) in the TCGA datasets, separated by patient sex (data visualization from www.cbioportal.org). Each dot represents one tumor; blue symbols have no mutation in the gene, and red have a mutation of the indicated type ($P<0.0001$ for all female-male expression comparisons by K-S test, either including or excluding mutated cases, see also Supplementary Figure 8). (**c**) M:F ratio of LOF mutations in the EXITS genes identified in Table 1, all other chrX escape (n=56), or chrX non-escape genes (data compared by t-test; bar represents median;'+', mean; box, interquartile range; whiskers,10–90%ile). (**d**) M:F ratio of LOF mutations in the EXITS genes that have functional Y homologs (*DDX3X, KDM5C*, and *KDM6A*), all other chrX genes with predicted functional Y homologs (n=14), or chrX genes without a Y homolog (data compared by t-test; plotted as in (c)).

Table 1

Genes with significantly (FDR<0.1) increased male (M) : female (F) mutation ratio identified by permutation analysis

Significance values are based on deviation of the observed mutation incidence in a specific gene relative to that expected in a given set. This approach normalizes to the number of male and female cancers (and to the number of X chromosomes), and to the background mutation incidence in male and female cancers in a given set.

Gene	Analysis set	LOF mutations	Total cancers	P value	Q (FDR) value
ATRX	all	70 M : 47 F	2440 M : 1686 F	0.000001	0.00066
ATRX	LGG	45 M : 19 F	98 M : 72 F	0.000001	0.000071
CNKSR2	all	30 M : 10 F	2440 M : 1686 F	0.00037	0.049
DDX3X	all	34 M : 9 F	2440 M : 1686 F	0.000026	0.0075
KDM5C	all	31 M : 10 F	2440 M : 1686 F	0.000092	0.015
KDM5C	KIRC	14 M : 1 F	216 M : 118 F	0.0003	0.044
MAGEC3	all	15 M : 1 F	2440 M : 1686 F	0.000034	0.0075
Gene	**Analysis set**	**LOF mutations or CN deletions**	**Total cancers**	**P value**	**Q (FDR) value**
KDM5C	all	24 M : 5 F	1225 M : 769 F	0.00022	0.079
KDM5C	KIRC	14 M : 1 F	216 M : 118 F	0.00047	0.08
KDM6A	all	50 M : 18 F	1225 M : 769 F	0.00025	0.079

LGG, lower-grade glioma; KIRC, clear cell kidney cancer; all, pooled data from all included cancer types; LOF, loss-of-function (see Methods); CN, copy number; FDR, false discovery rate.