What’s good for the goose would have been good for the gander: Sex differences in cancer.

I have no COIs to disclose

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Sex, Age, Anatomic Location, and Extent of Resection Influence Outcomes in Children With High-grade Glioma.
McCrea, Heather; MD, PhD; Bander, Evan; Venn, Rachael; Reiner, Anne; Iorgulescu, J; Puchi, Luis; Schaefer, Peter; Cederquist, Gustav; Greenfield, Jeffrey; MD, PhD

DOI: 10.1227/NEU.0000000000000845
• Sex differences in cancer
• Relationship between growth and cancer.
• Brief review of developmental and evolutionary origins of sex differences.
• Model for studying sex differences in cancer
• Sex specific responses to tumor suppressor loss
• Molecular basis for sex specific responses
• Clinical Implications
Throughout life there are sex-specific differences in the prevalence of disease

- Autism
- Stuttering
- Schizophrenia
- Pyloric Stenosis
- Parkinsons’ Disease
- Cardiovascular Disease
- Scoliosis
- Major Depression
- Neural Tube Defects
- Alzheimer’s Disease
- Autoimmune Disease
- Congenital Hip Dislocation
## Sex Differences in Cancer

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall cancer rate (per 10^5)</td>
<td>553</td>
<td>416</td>
</tr>
<tr>
<td>Cancer mortality rate (per 10^5)</td>
<td>223</td>
<td>153</td>
</tr>
</tbody>
</table>

### Ratio M:F cases for specific cancers

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal cancer</td>
<td>1.35</td>
</tr>
<tr>
<td>Lung</td>
<td>1.52</td>
</tr>
<tr>
<td>NHL</td>
<td>1.44</td>
</tr>
<tr>
<td>Bladder</td>
<td>4.0</td>
</tr>
<tr>
<td>Meningioma</td>
<td>0.125</td>
</tr>
</tbody>
</table>
Circulating sex hormones
Brain Tumors

Nat Rev Genet. 2008 9(12)

www.cancerresearchuk.org
Sex Differences in Brain Tumor Incidence

**United Kingdom** (Arora, et al. (2009))
Adult: MB(1.70); GBM(1.75); OLG(1.30); EPD(1.48)
Pediatric: MB(1.73); AST(1); OLG( 3.21); EPD(1.34)

**Germany** (Rickert, et al. (2001))
Pediatric: overall sex ratio (1.55)

**Sweden** (Lannering, et al. (2009))
Pediatric: MB (1.4); AST (1); EPD (1)

**Canada** (Tabori, et al. (2006))
Adolescent: MB (1.4)

**Morocco** (Karkouri, et al. (2010))
Pediatric: MB=1.2; GBM (1.5); OLG (3.5); EPD (3.5)

**US** (Hess, et al. (2004))
Adult: GBM (1.36); OLG (1.36)

(Kohler, et al. (2011))
Adult: GBM (1.58); OLG (1.36)
Pediatric: MB (1.33); PA (1.1); GBM (1.42); OLG (1.12)

**France** (Rigau, et al. (2011))
Adult: MB (1.39); GBM(1.46); OLG (1.31); EPD (1.3)

**USA**
(Hess, et al. (2004))
Adult: GBM (1.36); OLG (1.36)

(Kohler, et al. (2011))
Adult: GBM (1.58); OLG (1.36)
Pediatric: MB (1.33); PA (1.1); GBM (1.42); OLG (1.12)

**Brazil** (Rosemberg, et al. (2005))
Pediatric: overall sex ratio (1.17); first 2 years of life (2.15)

**Morocco** (Karkouri, et al. (2010))
Pediatric: MB=1.2; GBM (1.5); OLG (3.5); EPD (3.5)

**Israel** (Kushnir, et al. (2011))
Elderly adult (65+): MB=1.85; GBM=2.0; OLG=1.46

**Iran** (Mehrazin, et al. (2006))
Adult and Pediatric: MB=1.85; GBM=2.0; OLG=1.46

**Japan** (Nomura, et al. (2011))
Adult and pediatric: GBM (1.25); OLG (1.25); EPD (1.11); AST (1.11)

**Pakistan** (Ahmed, et al. (2007))
Pediatric: MB (3); PA (3); GBM (2); EPD (3)

**Italy** (Giordana, et al. (1999))
Adolescent and adult: MB (2.46)

**China** (Zhou, et al. (2008))
Pediatric: overall sex ratio (1.6);
MB (2.6); AST (1.3); EPD (1.3)

**Germany** (Rickert, et al. (2001))
Pediatric: overall sex ratio (1.55)

**Sweden** (Lannering, et al. (2009))
Pediatric: MB (1.4); AST (1); EPD (1)

**Canada** (Tabori, et al. (2006))
Adolescent: MB (1.4)

**USA**
(Hess, et al. (2004))
Adult: GBM (1.36); OLG (1.36)

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**Iran** (Mehrazin, et al. (2006))
Adult and Pediatric: MB=1.85; GBM=2.0; OLG=1.46

**Australia** (Dobes, et al. (2011))
Elderly adult with malignant brain tumors: (1.31)

Cancer is a disease a growth (and invasion, and destruction)

Does cancer risk relate to variations in normal growth?
Fig. 1 The relationship between the number of stem cell divisions in the lifetime of a given tissue and the lifetime risk of cancer in that tissue. Values are from table S1, the derivation of which is discussed in the supplementary materials.
Pre-implantation sexual dimorphism

Expression: G6PD, HPRT1, XIAP
Phenotype: Male 2 day embryo is bigger than the female

Expression: DNMT3A and B
Phenotype:
- M > F glucose utilization
- M > F lactate production
- F > M PPP activity

- Differential expression of X-encoded alleles (G6PD, OGT)
- Greater mitochondrial content in males
- Differences in epigenetic writers and erasers (OGT, UTX)
- Sex specific reprogramming of imprinted alleles
Post-implantation

Gonadal Development

ORGANIZING ACTION OF PRENATALLY ADMINISTERED TESTOSTERONE PROPIONATE ON THE TISSUES MEDIATING MATING BEHAVIOR IN THE FEMALE GUINEA PIG

CHARLES H. PHOENIX, ROBERT W. GOY, ARNOLD A. GERAL AND WILLIAM C. YOUNG

Department of Anatomy, University of Kansas, Lawrence, Kansas

Placental Function

<table>
<thead>
<tr>
<th>Gene name</th>
<th>ΔCt female ± SE</th>
<th>ΔCt male ± SE</th>
<th>α-Value ± SE (α)</th>
<th>Ln 2α</th>
<th>LCL</th>
<th>UCL</th>
<th>p</th>
<th>Change in gene expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>5.12 ± 0.80</td>
<td>5.78 ± 0.60</td>
<td>-0.66 ± 0.46</td>
<td>0.63</td>
<td>-0.13</td>
<td>1.31</td>
<td>0.05</td>
<td>No change</td>
</tr>
<tr>
<td>IGF-2</td>
<td>2.79 ± 0.4</td>
<td>1.09 ± 0.70</td>
<td>1.70 ± 0.70</td>
<td>1.17</td>
<td>0.46</td>
<td>2.04</td>
<td>0.04</td>
<td>Overexpressed</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>6.11 ± 0.62</td>
<td>4.65 ± 0.53</td>
<td>1.46 ± 0.44</td>
<td>1.01</td>
<td>0.25</td>
<td>1.63</td>
<td>0.03</td>
<td>Overexpressed</td>
</tr>
</tbody>
</table>

UCL, upper confidence limit; LCL, lower confidence limit; n female preterm = 52, n male preterm = 48.
Height and cancer risk
Chapter Four: “Thus it is, as I believe, that when the males and females of any animal have the same general habits of life, but differ in structure, colour, or ornament, such differences have been mainly caused by sexual selection; that is, individual males have had, in successive generations, some slight advantage over other males, in their weapons, means of defence, or charms; and have transmitted these advantages to their male offspring.”
Sex differences in growth regulation have been selected for by evolution

For both males and females reproductive success requires the passing of genes to their progeny and then on to their grand-progeny. This creates a fulcrum on which evolution works to maximize individual reproductive success and the successful growth of progeny until they achieve reproductive maturity.

<table>
<thead>
<tr>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>• significant</td>
<td>• less significant</td>
</tr>
<tr>
<td>• small</td>
<td>• Huge</td>
</tr>
<tr>
<td>Intrasex competition</td>
<td>Resource sacrifice/progeny</td>
</tr>
</tbody>
</table>

This creates conflict between males and females in growth regulation.
Implications for cancer biology: compensation for perturbations in growth

Female growth optimum

Male growth optimum

Male biased action

Female biased action

Crespi and Summers
RB and p53 pathway are at the core of growth regulation and cancer biology
Sex differences in response to combined loss of Nf1 and p53 function

Sun et al. JCI 2014
Sex differences in \textit{Nf1-/-;Dnp53} astrocyte clonogenicity

Male                 Female

Sun et al. JCI 2014
Sex impacts on the response of $Nf1^{-/-};DNp53$ to EGF

Sun et al. *JCI* 2014
Sex differences in response to loss of p53 function

Male cells \((Nf1^{-/-};DNp53^-/-)\)

Female cells \((Nf1^{-/-};DNp53^-/-)\)

Male Recipient Mice

Female Recipient Mice

→ Male \(Nf1^{-/-};DN-p53\) tumor

→ Female \(Nf1^{-/-};DN-p53\) tumor

Sun et al. JCI 2014
Sex differences in response to loss of p53 function

(P<0.0001)

Sun et al. JCI 2014
Sex specific drug effects

PROCESS FLOW CHART

Cell lines: M8 and F8
FDA approved drugs: 10 μM, 72h

ImageXpress Readout: Nuclei (DAPI) & Whole well GFP
Biotek Synergy Neo: GFP Reads

Data Analysis: Nuclei and Total GFP, % Inhibition

Cherry-pick 285 compounds (inhibition in M8 and F8)

Dose-response: 40, 20, 10, 5, 2.5, 0 μM
72h

Cell Titer Glo

Aspirated

Cell Titer Glo

Aspirated

ImageXpress

GFP plate reader

96 compounds cherry-picked for reproducibility

Not-Aspirated

Not-Aspirated

GFP plate reader

Image Analysis

25 FDA APPROVED / BIOACTIVES
Everolimus

GFP Biotek | Nuclei ImageXpress | GFP ImageXpress
--- | --- | ---

<table>
<thead>
<tr>
<th>Conc (uM)</th>
<th>% Inhibition</th>
<th>F</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>2.423</td>
<td>87.18</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>6.194</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>13.62</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CTG 24h | CTG 48h | CTG 72h
--- | --- | ---

<table>
<thead>
<tr>
<th>Conc (uM)</th>
<th>% Inhibition</th>
<th>F</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00001</td>
<td>9.161</td>
<td>86.97</td>
<td></td>
</tr>
<tr>
<td>0.0001</td>
<td>6.414</td>
<td>640.9</td>
<td></td>
</tr>
<tr>
<td>10000</td>
<td>4.481</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

Weir 2016

mTOR inhibitor

No inhibition at 100 uM
inhibition at 10 uM
Temsirolimus (mTOR inhibitor) inhibits cell growth in CTG cells at concentrations of 100 µM and 10 µM. The IC50 values are as follows:

- CTG 24h: IC50 6.097 µM
- CTG 48h: IC50 4.276 µM
- CTG 72h: IC50 4.567 µM

No inhibition at 100 µM and inhibition at 10 µM.
Sex differences in drug effects

Temozolomide

IC50 uM

Male
Female

Taylor 2016
Peto’s Paradox
There are evolutionarily selected and developmentally ensconced sex differences in growth regulation.

These may be related to sex differences in cancer rates and outcome.

Pathways with sexual dimorphism include the p53, RB and PI3 kinase pathways.

Sex differences in biology may be driven by differential super enhancer usage.

The biology of GBM is sex-specific.

Important sex differences are evident in therapeutic responses.
Acknowledgements

Rubin Lab
Najla Kfoury
Nicole Warrington
Sara Taylor
Nathan Rockwell
Jasmine Sponagel
Lauren Broestl
Inema Orukari
Cameron Hill
Julie Kenney

Wash U Collaborators
Rob Mitra
Will Yang
Rosy Luo
Sonika Dahiya

KU
Scott Weir
Melinda Broward
Anuradha Roy
Figure 1. Escape from X-inactivation tumor-suppressor (EXIT) 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 EXIT hypothesis. (b) ‘Traditional’ tumor-suppressor genes (TSGs) on the X chromosome 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 X chromosome and females have one active X chromosome (Xa; pink) and one inactive X chromosome (Xi; purple). (c) Model for EXIT gene behavior. In females, there are two active alleles of EXIT genes, and females are therefore protected from complete gene loss after a single alteration. Complete inactivation of an EXIT gene may require biallelic mutations, or mutation with loss of the other X chromosome. In males, one mutation could inactivate the only allele of an EXIT gene that has no functional Y-chromosome homolog, and males would therefore be more likely to develop cancers associated with mutations in those TSGs. Alternatively, because some genes that escape X-inactivation have Y-chromosome homologs with redundant function, cancers with mutations in those genes would be more likely to occur in males who also have somatic loss of the Y chromosome.
Figure 2 Genes with higher frequencies of somatic loss-of-function alterations in male cancers. (a, b) Permutation testing for genes on the X chromosome across all cancer data sets is shown. The log₂ (M:F) ratio of events is plotted for each gene against the significance (P) value. The size and color of each circle correspond to the number of loss-of-function mutations (a) or loss-of-function mutations and copy number loss events (b) in that gene. Genes with significantly higher (FDR < 0.1) frequencies of mutation in male cancers are labeled. (c, d) Disease-specific permutation testing of loss-of-function mutations in lower-grade glioma (LGG) (c) and clear cell kidney cancer (KIRC) (d).
Table 1  Genes with significantly (FDR < 0.1) increased M:F mutation ratios identified by permutation analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Analysis set</th>
<th>LOF mutations</th>
<th>Total cancers</th>
<th>P value</th>
<th>Q (FDR) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATRX</td>
<td>All</td>
<td>70 M: 47 F</td>
<td>2,440 M: 1,686 F</td>
<td>0.000001</td>
<td>0.000066</td>
</tr>
<tr>
<td>ATRX</td>
<td>LGG</td>
<td>45 M: 19 F</td>
<td>98 M: 72 F</td>
<td>0.000001</td>
<td>0.000071</td>
</tr>
<tr>
<td>CNKSRZ</td>
<td>All</td>
<td>30 M: 10 F</td>
<td>2,440 M: 1,686 F</td>
<td>0.00037</td>
<td>0.049</td>
</tr>
<tr>
<td>DDX3X</td>
<td>All</td>
<td>34 M: 9 F</td>
<td>2,440 M: 1,686 F</td>
<td>0.000026</td>
<td>0.0075</td>
</tr>
<tr>
<td>KDM5C</td>
<td>All</td>
<td>31 M: 10 F</td>
<td>2,440 M: 1,686 F</td>
<td>0.000092</td>
<td>0.015</td>
</tr>
<tr>
<td>KDM5C</td>
<td>KIRC</td>
<td>14 M: 1 F</td>
<td>216 M: 118 F</td>
<td>0.0003</td>
<td>0.044</td>
</tr>
<tr>
<td>MAGEC3</td>
<td>All</td>
<td>15 M: 1 F</td>
<td>2,440 M: 1,686 F</td>
<td>0.000034</td>
<td>0.0075</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Analysis set</th>
<th>LOF mutations or CN deletions</th>
<th>Total cancers</th>
<th>P value</th>
<th>Q (FDR) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>KDM5C</td>
<td>All</td>
<td>24 M: 5 F</td>
<td>1,225 M: 769 F</td>
<td>0.00022</td>
<td>0.079</td>
</tr>
<tr>
<td>KDM5C</td>
<td>KIRC</td>
<td>14 M: 1 F</td>
<td>216 M: 118 F</td>
<td>0.00047</td>
<td>0.08</td>
</tr>
<tr>
<td>KDM6A</td>
<td>All</td>
<td>50 M: 18 F</td>
<td>1,225 M: 769 F</td>
<td>0.00025</td>
<td>0.079</td>
</tr>
</tbody>
</table>

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) as well as to the background mutation incidence in male and female cancers in a given set. LGG, lower-grade glioma; KIRC, clear cell kidney cancer; all, pooled data from all included cancer types; LOF, loss of function (Online Methods); CN, copy number; FDR, false discovery rate.
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 fourfold male-biased loss-of-function mutations with Bonferroni-corrected P < 0.1 (that is, mutations with four times greater prevalence in tumors from males as compared to females). The x axis represents the fraction of all mutations on the X chromosome 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 specific mutations (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 on the basis of the fraction of mutations on the X chromosome in males and the number of tumor-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 cell carcinoma; KIRC, clear cell kidney cancer; KIRP, papillary kidney cancer; LAML, acute myeloid leukemia; LGG, lower-grade glioma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell 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 (log2) for DDX3X, KDM5C, and KDM5A in head and neck squamous cell carcinoma (HNSC) and clear cell kidney cancer (KIRC) in the TCGA data sets, with tumors separated by patient sex (data visualization from http://www.cbioportal.org/). Each dot represents one tumor: blue symbols are tumors with no mutation in the gene, and red symbols are tumors with a mutation of the indicated type (P < 0.0001 for all female–male expression comparisons by Kolmogorov–Smirnov test, either including or excluding mutated cases; see also Supplementary Fig. 8). Bar, median; box, interquartile range; whiskers, 10th–90th percentiles. Number of samples: HNSC, 203 male, 76 female; KIRC, 269 male, 144 female. (c) M:F ratios of loss-of-function mutations in the EXITS genes identified in Table 1, all X-chromosome escape genes (n = 56), and X-chromosome non-escape genes (data compared by t test; bar, median; plus sign, mean; box, interquartile range; whiskers, 10th–90th percentile). (d) M:F ratios of loss-of-function mutations in the EXITS genes that have functional Y-chromosome homologs (DDX3X, KDM5C, and KDM5A), all other X-chromosome genes with predicted functional Y-chromosome homologs (n = 14), and X-chromosome genes without a Y-chromosome homolog (data compared by t test; plotted as in c).