Do you know the sex of your cells?

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Shah K, McCormack CE, Bradbury NA. Do you know the sex of your cells? Am J Physiol Cell Physiol 306: C3–C18, 2014. First published November 6, 2013; doi:10.1152/ajpcell.00281.2013.—Do you know the sex of your cells? Not a question that is frequently heard around the lab bench, yet thanks to recent research is probably one that should be asked. It is self-evident that cervical epithelial cells would be derived from female tissue and prostate cells from a male subject (exemplified by HeLa and LnCaP, respectively), yet beyond these obvious examples, it would be true to say that the sex of cell lines derived from non-reproductive tissue, such as lung, intestine, kidney, for example, is given minimal if any thought.

After all, what possible impact could the presence of a Y chromosome have on the biochemistry and cell biology of tissues such as the exocrine pancreatic acini? Intriguingly, recent evidence has suggested that far from being irrelevant, genes expressed on the sex chromosomes can have a marked impact on the biology of such diverse tissues as neurons and renal cells. It is also policy of AJP-Cell Physiology that the source of all cells utilized (species, sex, etc.) should be clearly indicated when submitting an article for publication, an instruction that is rarely followed (http://www.the-aps.org/mm/Publications/Info-For-Authors/Composition). In this review we discuss recent data arguing that the sex of cells being used in experiments can impact the cell’s biology, and we provide a table outlining the sex of cell lines that have appeared in AJP-Cell Physiology over the past decade.

In 2001, The Institute of Medicine published a significant report highlighting the importance of sex as a variable in human and experimental studies (278). Over a decade later, the recommendations of this report have received meager acceptance. Most researchers acknowledge the importance of describing the sex of animals used in studies. In many cases only male animals will be used, in order to obviate any “complications” that may arise from hormonal differences in female animals during their reproductive cycles. Sex selection is obviously important in some studies, however. For example, it is obvious that a research study on milk production and lactation would be confined to male subjects. Despite the clear importance of knowing the sex when using whole animals, the basis for the development of new diagnostic and therapeutic interventions in human medicine. However, only half of the population may have a sex the same as the cell line on which the diagnostic test or treatment was developed. Since all cell lines have a “sex” (278), the complement of sex chromosomes has the potential to influence biochemical pathways and cell physiology (161). In this review, we provide a setting for the basis of differences between male and female cells and highlight why these differences will likely provide novel insights into the roles of the X and Y chromosomes. Throughout this review, we have avoided the use of the word “gender,” spe-
specifically referring to the “sex” of cells. According to Institute of Medicine, “sex” is a biological construct dictated by the presence of sex chromosomes and in animals and humans the presence of functional reproductive organs. On the other hand, “gender” is a cultural concept referring to behaviors that might be directed by specific stimuli (visual, olfactory) or by psychosocial expectations that result from assigned or perceived sex and therefore can influence biological outcomes (161, 278). This definition has now been accepted as a new policy for sex and gender in reporting research in all APS journals (http://www.the-aps.org/mm/hp/Audiences/Public-Press/For-the-Press/releases/12/9.html). Information on the sex of cell lines routinely used by authors of publications in AJP-Cell Physiology is also presented. Finally, we pose several questions that we hope will guide the scientific community with regard to the potential role of sex in studies using cell lines and at least cause researchers to consider the impact of the sex of a cell on the interpretation of experimental results.1

Males and Females Are Different

The first question to be asked is “is there any evidence of sex differences between male and female non-sexual tissue that cannot be explained by hormonal differences?” As physiologists, we all accept that there are obvious differences between males and females. In vertebrates, sex differences are usually attributed to the effects of embryonic and post pubertal hormones. Indeed, while many of the more obvious differences between male and female vertebrates are clearly dependent on hormones, the role of hormones in other tissues is much less certain. Aristotle, the ancient Greek philosopher and polymath, more than 2,000 years ago is purported to have articulated the notion that sexual dimorphism exists at the earliest stages of embryonic growth. He believed that male embryos became “animated” 40 days post conception, whereas female embryos required a further 50 days before becoming “animated” (4). Intriguingly, recent studies tend to support the notion of early differences between male and female embryos. For example, male embryos created through in vitro fertilization grow faster prior to implantation than female embryos (6, 199, 284). Importantly, these findings suggest that genetic cellular differences between sexes exist before the onset of hormonal exposure. Moreover, even in adults, hormonal ablation or supplementation does not completely eliminate or recreate sexual differences observed in the progression of certain tumors from male and female patients (38). Furthermore, pathologies that display a sex disparity, such as neurodegenerative (242, 299), cardiovascular (266), and autoimmune (16, 82, 140) disease, differ in frequency but not severity; a difference not readily explained by hormonal differences. Thus, it is clear that not every difference observed between male and female cells can be attributed to differences in exposure to sex hormones. Fundamental to the replication of chromosomes is the telomere, that short region of repetitive nucleotides at the end of each chromatid that protects the chromosome from deterioration or fusion with other chromosomes. The length of the telomere is shorter in older males compared with females (7, 270), leading to the postulation that differences in replicative rates affect telomere shortening and aging (253), and may explain why males die younger than females (236, 274).

Males Have a Y Chromosome

On a simplistic level, differences between male and female cells are entrenched in differences in genetic content, as expressed by the presence of sex chromosomes; two X chromosomes in female cells, and one X and one Y chromosome in male cells (Fig. 2). The role of the Y chromosome in male sex determination arose from observations that XY and XYY (Klinefelter syndrome) individuals develop testes whereas XX and XO (Turner syndrome) individuals instead develop ovaries (72, 104): note that individuals with Turner syndrome have so-called streak gonads located below the fallopian tubes and generally show no evidence of germinal elements (89). Thus while the presence of a single Y chromosome is necessary and
sufficient to generate a male gonadal phenotype, the presence of a single X chromosome, while necessary, is not sufficient to generate a full female gonadal phenotype. In 1990, the gene responsible for testicular determination, named SRY (sex-determining region Y) was identified (100, 111, 237, 245) (Fig. 2) and comprises a single exon encoding a 204 amino acid protein containing a DNA-binding domain (HMG-box: High Mobility Group), arguing for this protein as a regulator of gene expression. For many decades it was believed that the only role of the Y chromosome was the development of the male gonadal phenotype and the initiation of male fertility (251). This opinion was reinforced by the dearth of other obvious phenotypes that segregated with the Y chromosome; “hairy ears” being one of the few well-documented exceptions (59). However, this concept of the Y chromosome as a genetic wasteland is now being challenged (180, 210). Indeed, since the sex chromosomes account for 5% of the total human genome (1,000–2,000 genes on the X chromosome and <50 genes on the Y chromosome; 129, 224, 246), there is at least the mathematical possibility that 1:20 proteins (and related biochemical reactions and pathways or cell biological processes) would differ between males and females. Given such odds, it is hard to imagine that cells from males and females would not differ in at least some aspects of cellular biochemistry and physiology. The Y chromosome has two genetically distinct aspects (Fig. 2). The distal part of the short arm of the Y chromosome is shared with the most distal part of the short arm of the X chromosome and can recombine with its X chromosome counterpart during meiosis in males, a region termed the “pseudoautosomal region” because loci in this region undergo recombination during spermatogenesis, akin to homologous recombination between autosomes (98, 215). A second pseudoautosomal region is also present on the distal portion of the long arms of the sex chromosomes (74). The remainder of the Y chromosome does not undergo recombination with the X chromosome and strictly comprises Y chromosome-specific DNA. Compared with other chromosomes, the Y chromosome has a limited number of genes. The roughly two dozen different genes encoded on the Y chromosome can be divided into two categories. One cohort of Y chromosome-specific genes is expressed exclusively in the testes and is likely involved in germline development and spermatogenesis; mutation or deletion of some of these genes leads to male infertility (113, 129, 154). A second group of Y chromosome genes consists of genes that do have homologous counterparts on the X chromosome but may yield slightly different final protein products (275). For example, the gene on the Y chromosome encoding the ribosomal protein S4 (RPS4Y; Fig. 2), a component of the 40S subunit, results in a slightly different protein than that expressed on the X chromosome (RPS4X) with a 19 amino acid difference between the two sex-distinct proteins (66, 275). While functionally equivalent isoforms of ribosomal proteins exist in yeast, these differ by no more than a few amino acids (279), the 19 amino acid difference between “male” and “female” ribosomes suggesting the possibility of differences in ribosomal function between “male” and “female” cells. Similarly, nucleotide sequence analysis of the ZFY (zinc finger protein) shows it to be similar but distinct (383 amino acids of 393 are identical) from its X chromosome (ZFX) counterpart (233) (Fig. 2). The differences or similarities between other homologous proteins remains to be determined. However, since RPS4Y and ZFY are present only in males, it is possible that such “male”–specific expression can result in potentially extensive biochemical differences between “male” and “female” cells. Regardless of whether or not genes on the Y chromosome, other than SRY, are important in determining cellular function, the SRY genes certainly are. In the 45-day-old 46XY human fetus, these genes cause the gonadal ridge to develop into the testes (89). The fetal testes secrete Mullerian inhibiting hormone, which causes the regression of primordial Mullerian ducts; thus the fallopian tubes and uterus do not develop. The fetal testes also secrete testosterone, causing the differentiation of the primordial Wolffian duct system into the epididymis and vas deferens.

**Females Have Two X Chromosomes and No Y**

In contrast to male genomes that have only one X chromosome, female genomes have twice the amount of X chromosome genetic material compared with males. Thus, whereas females can be either homozygous or heterozygous with respect to X chromosome-linked traits, males (due to the presence of only one X chromosome) are hemizygous. Products of the X chromosome genes, like those of autosomes, are involved in many aspects of cellular function, metabolism development, and growth (224). Indeed, the X chromosome contains the largest number of immune-related genes within the entire genome (19). In contrast to males where genes from only one X chromosome are present, the occurrence of two X chromosomes in females gives rise to the potential expression of twice the amount of X chromosome DNA in females compared with males. This double dosage of X chromosome genes in females is, however, annulled at many loci by the process of X chromosome inactivation (39, 167, 196, 283). This fundamentally female process is never found in normal XY males (89) and only occurs in female cells outside of the germline. The process of X inactivation profoundly alters the cell’s transcriptional landscape, engendering epigenic changes and differential nuclear compartmentation of chromosomes in a highly regulated fashion (97). The inactive chromosome changes conformation to yield a darkly staining mass called the sex chromatin or Barr body (143). Because of the random nature in the choice of which of the two X chromosomes are inactivated (206), females have two epigenetically distinct populations of cells, in which either the maternally or the paternally derived X chromosome is expressed (196). Males, by contrast, only express an active maternally derived X chromosome in all cells; of course the “maternally derived” X chromosome could itself be paternally derived. The random feature of X chromosome inactivation leads to a mosaic of expression of the two X chromosomes in female tissues, and this has been invoked as the basis for lack of a tight genotype-phenotype correlation in the severities of recessive X chromosome-linked diseases (156). A classic example of random X-inactivation is presented by the calico, or tortoiseshell, cat. Each X chromosome expresses either an orange or a black coat coloring, yet the calico cat coat pattern is extremely common. This illustrates the fact that both X chromosomes contribute to the cat’s color and explains why almost all calico cats are female (181). Since males only have a single X chromosome, “variants” in genes on one X chromosome cannot be obviated by a second X chromosome. Thus, males demonstrate a
Clearer, more common or more extreme version of any variant phenotype than females do. This is exemplified at its extreme by “X-lined” genetic diseases, including Duchenne and Becker muscular dystrophies (168), hemophilia (24), porphyria (3) X-linked cone-and rod-dystrophies (160), and color blindness (174). A dramatic example of male hemizygosity for X chromosome-linked traits is seen in X chromosome-linked dominant mutations. Mutations in these genes are embryonically lethal to males in utero and are therefore only seen in females. For example, X chromosome-linked incontinentia pigmenti is a relatively benign dermatological condition in females, but it is lethal to males who inherit a mutant allele (249).

Intriguingly, in females there are reports of a strong somatic selection against cells that bear mutations on the active X chromosome (17, 277). For example, the B-cell lineage in heterozygous females carrying mutations at the X chromosome-linked agammaglobulinemia show selective inactivation of the mutant chromosome and expression of genes from the non-mutant X chromosome (45). Despite the process of X-chromosome inactivation, not all genes on the X chromosome are subject to inactivation (55, 277, 289). As much as 15% of X chromosome-linked genes have been identified as being expressed from the “inactive” X chromosome in at least some cells in culture (34, 35). A notable example of this is seen in the ZFX gene (185, 233) (Fig. 2), a zinc finger protein expressed only on the X chromosome and therefore completely absent from males. Moreover, some genes are transcribed with equal efficiency from both the “active” and “inactive” chromosome. For example, gastrin-releasing peptide (GRP) is known to be expressed by both the active and inactive X chromosomes. More than a curiosity, this double expression of GRP may have important clinical consequences, as elevated levels of GRP are proposed to be associated with an elevated risk of lung cancer in women who smoke (241).

Male and Female Cells Are Not the Same

Nearly all biochemical, signaling, and trafficking pathways elucidated for mammalian cells have been obtained from studies on cell lines. Some of these cell lines have been cultured for over 50 years and were considered for their functional and morphological features without regard to their sex origin. A notable exception is the HeLa cell, which is the oldest and probably most widely used of all cell lines. Obtained from a patient with cervical cancer, the cells were taken without consent from Henrietta Lacks, a female patient at Johns Hopkins hospital, who eventually died of her cancer on October 4, 1951 (78, 106, 231). Indeed, the sex of the HeLa cell is fairly well known even to the general public thanks to a recent best seller in the popular science press (247). HeLa cells have been central to many biomedical breakthroughs of the last half century, from their initial use in the development of a polio vaccine (231) to their key role in studies leading to the awarding of two Nobel Prizes in Physiology or Medicine: Harald zur Hausen, in 2008, for his discovery of human papilloma viruses causing cervical cancer (26), and Elizabeth Blackburn, Carol Greider, and Jack Szostak, in 2009, for their discovery of how chromosomes are protected by telomeres and the enzyme telomerase (231, 239, 265). More recently, HeLa cells have again gained prominence as drivers of National Institutes of Health (NIH) policy. In April 2013, a group working at the European Molecular Biology Laboratory in Heidelberg, Germany, published the genome of the HeLa cell line (130). At the same time, an NIH-funded group working at the University of Washington was preparing to publish their version of the HeLa genome (1). Given that immediate descendants of Henrietta Lacks are still alive, concern was raised by other researchers and by the Lacks family that the genome sequence could reveal heritable aspects of Lacks’ germline DNA. Such sequence data could be used to draw inferences concerning the Lacks family’s medical status, engendering a quagmire of legal and ethical issues. NIH has now implemented a new policy regarding the distribution and use of genome sequence data from HeLa cells (grants.nih.gov/grants/ guide/notice-files/NOT-OD-13–099.html). Under the new guidelines, the DNA sequence data from HeLa cells will be subject to controlled use; applications to access the sequence data are being reviewed by a newly formed HeLa Genome Data Access working group at NIH, on which two members of the Lacks family will serve. The difficulty of the HeLa cell has, unfortunately, also proven to be one of its greatest concerns. HeLa cells have been noted to contaminate and indeed overgrow other cell cultures grown within the same laboratory, interfering with, and invalidating, many publications. The degree to which HeLa cell contamination is a problem remains unknown, as few researchers have the time, money, or knowledge for determining the purity of cell lines within their laboratories. However, contamination by HeLa cells has been estimated to range between 10% and 20% of all cell lines in use (150), and cross-contamination remains a major ongoing problem in modern cell cultures (32, 173). Despite these concerns, cell lines are vital to much of current biomedical research. The advances in basic biomedical sciences, and in the development of pharmacological treatments for numerous diseases, would not be possible without the use of cell lines obtained from human and non-human sources. As scientists, we owe a great debt to those patients who have willingly and unwittingly provided the tissue samples upon which so many of us rely for our research.

Differences between the male and female brain have been a subject of study by philosophers, poets, and scientists alike. It has long been held that sex differences in the brain are caused by differential exposure to gonadal secretions during fetal and neonatal development (5), with distinct sexual dimorphism particularly in sex steroid-concentrating regions (145). However, there is accumulating evidence that supports the notion of sexual dimorphism in the brain in the absence of gonadal secretions (202, 218). For example, morphological and functional sex differences in dopaminergic (and probably noradrenergic) neurons are seen in cultures of rat brain tissues removed at day E14 (day of insemination = E0), whereas the male rat gonad does not start to secrete testosterone until day E15 (217). In fact, no measurable differences in whole body androgens are seen in rats until after E18. Many of the differences in brain-derived cells are retained even following growth of excised tissues, from male and female brains, in identical culture media. Studies by Dewing et al. (53) have described over 50 different sex-dimorphic genes, i.e., genes that display intrinsic differences between male and female cells that are not dependent on hormone exposure and persist in cell culture. Dopaminergic neurons, although accounting for less than 1% of brain neurons, are nonetheless critical for such...
Sex diversity of gene expression is not reserved for the CNS alone, however. For example, kidney cells obtained from female embryonic rats are significantly more sensitive to ethanol- and camptothecin-induced apoptosis than their male counterparts (197). While male and female splenocytes display similar responses to nitrosative stress and staurosporine-induced apoptosis, female splenocytic cells are more sensitive than their male counterparts and react to significantly lower doses of staurosporine than male cells (60). Cyp1A1 is a member of the cytochrome P-450 family, a family of proteins responsible for the metabolism and inactivation of many drugs and toxins (211, 272). Cyp1A1 plays a particularly prominent role in the metabolism of polycyclic aromatic hydrocarbons present in cigarette smoke. Female smokers have a higher level of aromatic/hydrophobic DNA adducts in lung tissue than males, due to a more responsive Cyp1A1 enzyme (166). High levels of lung DNA adducts have been related to an early onset of lung cancer (228), and several, though not all, epidemiological studies have suggested that with similar exposure to cigarette smoke, females may be at greater risk of developing lung cancer than males (295). Differences in drug metabolism are also seen in male and female livers. Female liver cells have more cytochrome CYP3A compared with male liver cells (186). Again, more than just a biochemical curiosity, such differences in CYP3A expression between male and female hepatocytes have important clinical consequences, as the actions of CYP3A account for the metabolism of half the drugs in the pharmacopeia (261, 296). Thus, for 50% of prescription drugs, the effectiveness of a particular drug dosage of 50% may be quite different in females compared with males (90).

A recent attempt to catalog differential gene expression between male and female cells examined 233 lymphoblastoid cell lines: 115 female and 118 male lines. Utilizing 4,799 probes, 10 autosomal genes were identified as having a sex-specific expression pattern (298). These genes encode a wide variety of proteins involved in multiple cellular processes, including cell adhesion, apoptosis, zinc ion binding, transcription factors, and structural molecules. When such studies are extended to more tissues, it appears that thousands of genes may show sexually dimorphic gene expression (290). Microarray analysis of 23,574 transcripts from murine liver, adipose, muscle, and brain tissues showed highly tissue-specific patterns of sexually dimorphic gene expression (290). The degree of sexual dimorphism ranged from ~35% in brain to ~70% in liver, likely (at least in part) accounting for the differential drug metabolism observed between males and females (2). Given such differences in gene expression, the question arises as to whether such differences result in a physiological phenotype. Stem-cell mediated muscle regeneration in mouse models of muscular dystrophy has raised some interesting data related to this point (51). Female muscle-derived stem cells are less sensitive to oxidative stress and regenerate skeletal muscle much more efficiently than muscle-derived stem cells from their male counterparts when transplanted into mdx or mdx/SCID mice, a dystrophin-deficient animal model of muscular dystrophy (51). Precisely how these differences arise is not immediately apparent, although differences in handling of oxidative stress appear to be a key feature between male and female cells. The finding that male and female muscle-derived stem cells have different properties is likely to have a big impact on other stem cell-mediated therapies should the findings be replicated for other diseases. Although the molecular mechanisms and genes involved in the sex disparity observed across various cell types await a fuller elucidation, what does seem to be a recurrent theme is the observation that female...
What Sex Are My Cells?

The notion that there are sex differences between cells has gained prominence through the increased use of primary cells obtained from both animals and humans. For animal studies, the sex of the primary cells can be known without difficulty, though IRB restrictions usually preclude immediate knowledge of the sex of the patient when cells are derived from human tissues. In contrast, the sex of cultured cells has been rarely considered (161, 260; Fig. 1). Indeed, while cultured cell lines have provided a plethora of data on biochemical pathways, cell biological processes, and gene expression, they have essentially been considered asexual objects of study. To facilitate the inclusion of the cells’ sex in future manuscript submissions to the AJP-Cell Physiology, Table 1 was generated by examining the last decade of papers published in AJP-Cell Physiology. Although clearly not an exhaustive list, it does represent the majority of cell lines used by submitting authors. Any omission of cell lines is the responsibility of the authors of this review and was completely unintentional. While the sexes presented in Table 1 are based on published data, it is also possible that some researchers may have worked with contaminated or misidentified cell lines (41), including incorrect sex assignment (63).

Yet, how can sex be determined for a cell line? In the modern era of molecular genetics, determination of the sex of a cell line utilizes an identical approach to that taken by forensic pathologists in determining the sex of human remains. Sexing cells by polymerase chain reaction (PCR)-based methodology is accomplished by amplification of homologous genes found on the X and Y chromosomes. The amelogenin gene is one such gene, and it codes for an extracellular matrix protein found in the developing tooth (64, 230). In humans, it has been determined that there are two amelogenin genes, one on the X chromosome (in the p22 region of the short arm; 156) and the other in the pericentric region of the Y chromosomes (132, 172, 230). Nakahori et al. (172) demonstrated the presence of a 6-bp insertion in intron 1 of the amelogenin-Y sequence (Y chromosome) that was absent from the amelogenin-X (X chromosome) gene. This 6-bp insertion results in a size difference between PCR products covering the intron-1 region, and it has been used to differentiate males from females (91, 147). Since both males and females have at least one X chromosome, the PCR product derived from the X chromosome is, automatically, a positive control. Separation of the “male” and “female” PCR products can be achieved by gel electrophoresis or denaturing high-performance liquid chromatography (240). Thus, females will show a single band for the amelogenin-X gene, whereas males will have two bands, one corresponding to the amelogenin-X gene and one from the male amelogenin-Y gene (Fig. 3). Furthermore, since the area under the curve can be used to quantify the amount of PCR product, it is also possible to identify XXY (Klinefelter syndrome) and XYY DNA. Amelogenin-based sex tests are part of various PCR multiplex reaction kits from different manufacturers and are widely used for DNA typing for samples in the forensic field (29).

While the determination of amelogenin gene expression should be relatively straightforward, there are a few cases where sex assignment based on this assay has not aligned with classic cytogenetic analysis of metaphase chromosomes. For example, cell line ATTC CRL-5873 (NCI-H1514), established in October 1986 from a 56-yr-old female with small cell lung carcinoma, shows positive for the Y chromosome-specific amelogenin sequence (63). Exactly how Y chromosome-specific PCR products end up in a female cell line is not entirely clear, although the possibility exists that there has been a misidentification of the cell line (http://www.atcc.org/Products/Cells%20and%20Microorganisms/Cell%20Lines/Misidentified%20Cell%20Lines.aspx). As can also be seen in Table 1 (noted by asterisks), some cell lines display an amelogenin test result consistent with a female genotype, yet the tissue of origin is from a documented male donor. For example, the PC-3 cell line is derived from human prostate epithelial cells (arguably an exclusively male tissue type), yet this cell line lacks the amelogenin-Y gene consistent with a male genotype. Indeed, over 100 reportedly “male” cell lines in the ATCC collection appear to have lost all trace of their Y chromosome and yield only X chromosome amelogenin during analysis (190) (Table 1). For example, in 1990, the cell line CRL-2234 was isolated from a hepatocellular carcinoma from a 52-yr-old Asian male (188). According to ATCC and “Short Tandem Repeat” (STR) analysis, CRL-2234 cells characteristically have a low amelogenin-Y peak, which decreases with passage. By passage 17, the Y chromosome can no longer be detected by routine amelogenin analysis. Whether other Y chromosome-specific genes are also lost with passage of CRL-2234, or indeed other “male” cell lines, is not known. Such loss of the Y chromosome, of course, severely impedes assessment of sex chromosome function on cellular functions. Several intestinal epithelial cell lines, including T84 (derived from a colonic carcinoma, isolated by H. Masui from a metastatic site in the lung from a 72-yr-old male patient; 54) and HT29 (isolated in 1964 by J. Fogh from a colonic cancer in a 44-yr-old female Caucasian; 271), have been extensively employed both by electrophysiologists and by cell biologists studying transepithelial ion/solute transport and polarized protein trafficking. Moreover, several studies have compared the biology of these cell lines (33, 40, 44). Since T84 colonic epithelial cells are derived from a male patient and HT29 colonic epithelial cells are derived from a female patient, one would think that the T84 and HT29 cell lines would be an ideal pair to discern any male/female differences in epithelial biology. However, when direct comparisons are made within the same study, little difference has been noted between T84 and HT29 cells. However, data from ATCC reveals that T84 cells have lost their Y chromosome (as detected by amelogenin analysis). Thus, whether similarities between T84 and HT29 cells are due to a biology exclusively related to autosomal gene expression, or whether differences would exist had T84 cells retained their Y chromosome, is difficult to evaluate.

Sex Disparity

Many sex disparities in disease severity and prognosis have been ascribed to hormonal differences. It will be interesting to
<table>
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<th>Cell Line</th>
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<th>Year</th>
<th>Origin</th>
<th>Reference</th>
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<td>1974</td>
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<td>(68, 69)</td>
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<td>(84, 85)</td>
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<td>Human</td>
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<td>N</td>
<td>(46, 38)</td>
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<td>Human</td>
<td>1972</td>
<td>C</td>
<td>(79, 137)</td>
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<tr>
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<td>Xenopus</td>
<td>1965</td>
<td>N</td>
<td>(165, 222)</td>
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<td>1976</td>
<td>N</td>
<td>(115, 183)</td>
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<td>Human</td>
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<td>C</td>
<td>(10, 11)</td>
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<tr>
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<td>N</td>
<td>(61, 282)</td>
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<td>Human</td>
<td>1987</td>
<td>C</td>
<td>(121, 229)</td>
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<td>1977</td>
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<td>1990</td>
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<td>N</td>
<td>(81)</td>
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<td>CPAE</td>
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<td>1965</td>
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<td>1994</td>
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<td>1987</td>
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<td>1976</td>
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<td>Mouse</td>
<td>1981</td>
<td>N</td>
<td>(273)</td>
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<td>1970</td>
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<td>1977</td>
<td>C</td>
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see how many of these differences really are hormonally mediated and which arise from intrinsic differences in male and female cells that are unrelated to hormonal exposure. However, for human tissues, such experiments are technically difficult, as testes in male fetuses start to develop by weeks 6 to 8. Studies are beginning to elucidate sex differences in gene expression levels and phenotypic responses; many of these, however, are still at the descriptive level. It will be important to define the precise mechanistic underpinnings of these observations of differences between male and female cells and how these observations may impact on cells that are maintained in cell culture. In 1993, the NIH mandated enrollment of women in human clinical trials, yet no similar initiative has been put forward for research using animals. As a result, male-to-female bias in neuroscience research studies has been estimated to be around 5.5:1 for male:female animal studies (15). The reasons for this disparity are likely varied but are mostly based on the assumption that results from males apply to females, or because the presence of hormonal cycles will increase the variance in acquired data, confounding interpretation of data (177, 278). However, based on data presented in this review it is clearly inappropriate to assume that results from studies conducted on only one sex will apply wholesale to the other (182).

Future Perspectives

We are now entering an era of physiological genomics and individualized medicine. It is clear that the presence of an XY or XX chromosome pair will have an impact on how an individual will respond to, or metabolize, a particular drug regimen (2). Many pharmaceutical companies and university research labs are developing high-throughput screening assays to identify and develop drugs for the treatment of various human diseases. Not only are cell lines being utilized, but also...
primary cells have a growing part in drug screening. The question arises, should we screen on male cells? Female cells? Or both? Even when it is known that there is a sex disparity in disease severity, this issue is rarely raised. For example, it is known that girls with cystic fibrosis do not grow as well as boys and have poorer lung function (223); under the age of 20 there is a 60% greater chance of girls dying compared with boys (52). The development of primary human airway cells as a model for cystic fibrosis has been a huge boon for the discovery and development of the first FDA-approved drug for the treatment of a subpopulation of patients with cystic fibrosis; yet none of the published reports has provided any information on the sex of the patient from whom the airway cells were obtained (267). A similar dearth of sex data is seen in other reports on the identification and development of other drugs for the treatment of patients with cystic fibrosis (268, 269). Such omissions, however, are not solely at the discretion of the researcher. IRB protocols prohibit the disclosure of any data that may lead to patient identification. Thus for compliance, researchers are generally not given access to such data. It can be reasonably argued that it is now time to release the restriction on revealing the sex of tissues used in drug screening. Although the sex of the cells being used in drug screens could be independently determined through amelogenin determination, this is both duplicative of existing data and may be construed as attempts at patient identification. It is clear that IRB members should at least appraise themselves of the importance of researchers knowing the sex of tissues as they develop screening assays. As noted earlier, muscle-derived stem cells from female mice regenerate muscle tissue when transplanted into dystrophic (mdx) mice to a greater extent than muscle-derived stem cells from male mice (51). In addition, in mdx mice, female hosts exhibit a significantly higher regeneration than male hosts (51). Whether this effect will be seen with human muscle-derived stem cells is not known. Muscle-derived stem cells can also undergo osteogenic differentiation with BMP4 treatment in vitro (128, 281). When male muscle-derived stem cells were used to evaluate ectopic intramuscular bone formation, male hosts (unaltered or castrated males) showed significantly more bone formation than when the same male stem cells were placed in female hosts (unaltered or ovarioctomized) (157). Thus clearly, not only does the sex of the stem cell matter, but also the sex of the host into which the cells are placed. Should stem cells prove useful for the treatment of patients with dystrophies or compromised bone wound healing, the sex of the donor and recipient will have an impact on patient prognosis, raising questions of survival and function of cell grafts in the same- and opposite-sexed recipients.

Can those of us who predominantly work with cultured cells escape the impact of the sex of our cells? With few exceptions, cells are cultured in media containing serum, although some manufacturers supply media to be used without serum. Certainly, unless specifically removed, such media will contain sex steroids. What is the impact of these steroids when culturing cells of unknown or indeed known sex? The matter is further complicated if one is utilizing “male” cells that have lost their Y chromosome (Table 1). While the issue of how representative is the biology of a cell line with respect to the tissue from which it was obtained is one with which most researchers are keenly aware, the potential impact of the loss of an entire chromosome on a cell’s biology is seldom considered. It is now perhaps time that such changes are contemplated. Although it is premature (and probably unrealistic) to suggest that all studies be performed on a cohort of both male and female cells prior to publication, the notion that there may be male/female differences in experimental outcomes is clearly not something that should be dismissed out of hand.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

K.S. prepared figures; N.A.B. drafted manuscript; C.E.M. and N.A.B. edited and revised manuscript; N.A.B. approved final version of manuscript.

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