Am J Physiol Cell Physiol 306: C3–C18, 2014.
First published November 6, 2013; doi:10.1152/ajpcell.00281.2013.

Review

Do you know the sex of your cells?

Kalpit Shah, Charles E. McCormack, and Neil A. Bradbury

Department of Physiology and Biophysics, Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, Illinois

Submitted 11 September 2013; accepted in final form 31 October 2013

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.

amelogenin; cell line; sex; X chromosome; Y chromosome

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 utilize only female animals, whereas studies on spermatogenesis would be confined to male subjects. Despite the clear importance of knowing the sex when using whole animals, such sex assignments are paid scant attention when studies are performed using cell lines (Fig. 1). After all, cells derived from male and female organisms display the same general characteristics. Cells derived from both sexes support metabolic processes, proliferate, and undergo differentiation. Cells, whether they are obtained from a male or female, possess a nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, and other cellular organelles. The assumption is made that, because there is really no difference in architecture or function between cells from male and female organisms, the Instructions to Authors (when submitting to the *AJP-Cell Physiology*), which state that the source of all cells utilized (species, sex, etc.) should be clearly indicated, can be happily ignored. A survey of a recent issue of *AJP-Cell Physiology* revealed that only two articles referenced the sex of the animal used, and none referenced the sex of the cell lines employed. Even when including a larger sample size, 75% of all recent publications in *AJP-Cell Physiology* did not discuss the sex of cell lines or animals used in the investigations (Fig. 1). Such omissions are not peculiar to *AJP-Cell Physiology* though. A recent review of publications describing the use of cultured cells in cardiovascular studies found a similar paucity of information on the sex of the cell lines utilized (260). Why is the sex of cell lines used in studies so often omitted from the final published article? It is likely that the sex of the cells being used was simply not known by the investigators, who, like most of us, simply regard the sex of our cells as irrelevant. The utility of cultured cells in identifying biological mechanisms, pathways, and processes is beyond doubt. Indeed, the results from such studies are often 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-

Address for reprint requests and other correspondence: N. A. Bradbury, Dept. of Physiology and Biophysics, Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064 (e-mail: neil.bradbury@rosalindfranklin.edu).

0363-6143/14 Copyright © 2014 the American Physiological Society

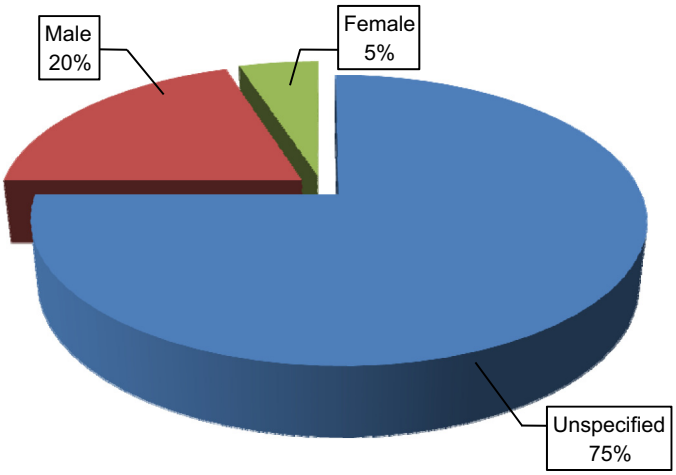

Fig. 1. Distribution of studies by sex, published in *AJP-Cell Physiology* in 2013. Shown is the percentage of articles describing the sex of cells derived from male subjects, female subjects, or unreported ($n = 100$ articles randomly selected from *AJP-Cell Physiology* manuscripts published in 2013).

cifically 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

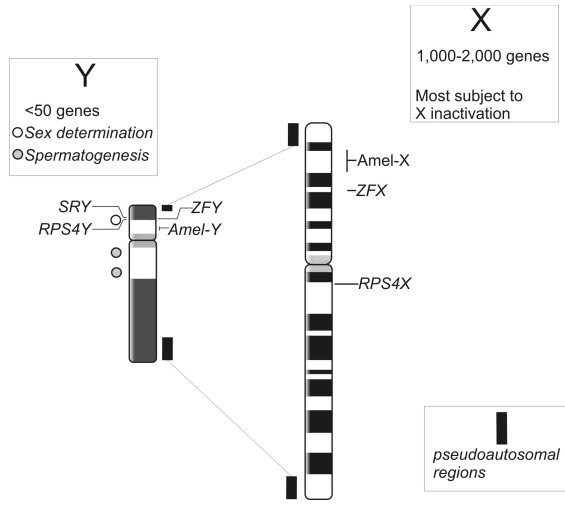

Fig. 2. Comparison of size and gene organization for X and Y chromosomes. Approximate locations of chromosome-specific genes for zinc finger proteins (*ZFX* and *ZFY*) and ribosomal proteins (*RPS4X* and *RPS4Y*) are shown, as well as locations for chromosome-specific amelogenin (Amel) genes used for sex determination. See text for details.

[1] This article is the topic of an Editorial Focus by Catherine M. Fuller and Paul A. Insel (74a).

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 gonadal 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 *RP4SY* and *ZFY* are present only in males, it is possible that such "male"-specific expression can result in potentially extensive biochemical differences between "male" cells 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 hardiness 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 have 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 wittingly 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

diverse brain functions as voluntary movement (134, 263), stress response (135), and addictive behavior/reward (159, 220). Dopaminergic neurons from female rat fetuses, in dissociated cell cultures, are morphologically distinct from those obtained from male rat fetuses, differences that are present even when gonadal hormones are absent (36). Moreover, cultured female neurons display a dopamine uptake rate twice that of their male counterparts (217). Gene array studies using nigral dopaminergic neurons from male and female patients with Parkinson's disease (obtained post mortem by laser capture dissection) have shown considerable sex-specific transcriptional profiles (244). Sex dissimilarities were not confined to a specific pathway but displayed differential transcription patterns in signal transduction, neuronal maturation, protein kinases, proteolysis, and WNT signaling (31, 244). Results from such studies support the notion that being male is a risk factor for Parkinson's disease. Indeed, epidemiological studies have shown that both the incidence and the prevalence of Parkinson's disease are 1.5 to 2 times greater in males that females (144, 280). Furthermore, the age of onset of Parkinson's disease is slightly earlier (mean 2.2 years) in men than women (92).

The hippocampus plays a key role in both short- and long-term memory (133, 184), as well as spatial navigation (22). Cultured male hippocampal neurons survive longer under normoxic conditions than female-derived hippocampal neurons but are more sensitive to ischemia than their female counterparts (99). In Alzheimer's disease, the hippocampus is one of the first regions of the brain to be affected; women are disproportionately affected by Alzheimer's disease, with two thirds of all sufferers being female (37). It is interesting to speculate that the sex disparity observed between male and female Alzheimer's patients may have an underlying basis in genes differentially expressed from the X and/or Y chromosomes in hippocampal neurons. In addition to differences in sensitivity to oxygen tension between male and female cells from the hippocampus, differential sensitivity to a wide range of cytotoxic agents has been shown for several neurons of the central nervous system (CNS) (60). For example, neurons from male rats are more sensitive to nitrosative ($ONOO^-$) stress than those neurons obtained from female rats. In contrast, neurons from female rats are more sensitive to apoptosis-inducing agents (staurosporine and etoposide) than neurons from their male counterparts (60). These observations are relevant to many CNS pathologies, where nitrosative stress is thought to play an important role in cerebral ischemia and traumatic brain injury. At a biochemical level, this may be related to the observation that male neurons are unable to maintain high levels of the reductant glutathione (60), a key protector from oxidative insult (73, 109). Mitochondria from female rats contain higher glutathione peroxidase (a key enzyme in maintaining cellular glutathione levels) activity than those from males (25). Such differences between the ability of male and female neurons to respond to oxidative stress and ischemia may provide an underlying mechanism for the observation that boys have a worse outcome following traumatic brain injury compared with girls (58).

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 encoded 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 ~14% 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

cells are better able to survive stress than male cells. Given the broad range of stress responses, this could arise from multiple genes present on one or both of the X chromosomes in females.

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 quantitate 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 1. *Table of the most commonly used cell lines appearing in AJP-Cell Physiology*

Cell Line	Sex	Description	Species	Year	Origin	Reference
5637	Male	Urinary bladder epithelium	Human	1974	C	(68, 69)
3T3-L1	Male	Embryo fibroblast	Mouse	1962	N	(84, 85)
16HBE		Lung epithelial	Human		N	(46, 88)
A549	Male	Lung epithelial	Human	1972	C	(79, 137)
A6	Male	Kidney epithelial	*Xenopus*	1965	N	(165, 222)
A7r5		Aorta smooth muscle	Rat	1976	N	(115, 183)
AGS	Female	Stomach epithelial	Human	1979	C	(10, 11)
AML-12	Male	Liver epithelial	Mouse	1994	N	(61, 282)
AML-193	Female	Lymphoblast	Human	1987	C	(131, 229)
ARPE-19	Male	Retinal pigmented epithelial	Human	1986	N	(62, 101)
BeWo	Female	Placenta	Human	1966	N	(193, 194)
BHK		Kidney fibroblast	Syrian hamster	1961	N	(146, 148)
BT-549	Female	Breast epithelial (ductal)	Human	1978	C	(112, 139)
BW5147.3		T-lymphocyte		1973	C	(212, 213)
C2BBe1	Male	Colonic epithelial cell (a CaCo-2 subclone)	Human	1988	C	(13, 200)
C2C12		Myoblast	Mouse	1977	N	(285, 286)
C6		Glial cell	Rat	1968	N	(18, 138)
C127	Female	Mammary epithelial	Mouse			(141)
Ca SKI	Female	Cervical epithelial	Human	1977	C	(195, 291)
CaCo-2	Male*	Colonic epithelial	Human	1977	C	(14, 107)
Calu-3	Male*	Airway epithelial	Human	1975	C	(70, 94)
Capan-1	Male*	Pancreatic epithelial	Human			(70, 71)
CCL-39	Female	Fibroblast	Chinese hamster	1964	N	(214, 252)
CFPAC-1	Male	Pancreatic epithelial	Human	1990	C	(155, 235)
CHO	Female	Ovarian epithelial	Chinese hamster	1957	N	(110, 207)
COS-7		Kidney fibroblast	African green monkey	1964	N	(81)
CPAE	Female	Pulmonary endothelial	Cow	1979	N	(23)
CRL-2234	Male*	Hepatocyte	Human	1990	C	(189)
CV-1	Male	Kidney fibroblast	African green monkey	1964	N	(105, 117)
DDT1-MF-2	Male		Syrian hamster	1983	C	(178, 257)
DU 145	Male	Prostate epithelial	Human	1978	C	(187, 254)
ES-D3		Embryonic stem cell	Mouse	1985	N	(56, 57)
GH3	Female	Pituitary epithelial	Rat	1965	C	(8, 258)
GH4C1	Female	Pituitary epithelial	Rat	1968	C	(258, 259)
H441	Male	Lung epithelial	Human	1982	C	(77, 179)
H460	Male	Lung epithelial	Human	1982	C	(9, 30)
H4TG	Male	Liver epithelial	Rat	1964	C	(93, 203)
H9c2		Myocardial myoblast	Rat	1976	N	(28, 116)
H460	Male	Lung epithelial	Human	1982	C	(9, 30)
HEK293	Female	Embryonic kidney epithelial	Human	1977	N	(80, 83)
HEL299	Male	Lung fibroblast	Human	1982	C	(201, 226)
HeLa	Female	Cervical epithelial	Human	1953	C	(78, 106)
HEP 3B	Female	Liver epithelial	Human	1983	C	(119, 120)
HEP G2	Male	Liver epithelial	Human	1994	C	(119, 120)
Hepa 1–6		Liver epithelial	Mouse	1987	C	(48, 49)
HET-1A	Male	Esophageal epithelium	Human	1986	N	(162, 255)
hFOB	Female	Osteoblast	Human	1997	N	(95, 96)
HK-2	Male	Kidney epithelial	Human	1994	N	(227)
HL-60	Female	Premyeloblast	Human	1979	C	(75)
HOS	Female	Mixed osteoblast/epithelial	Human	1975	C	(153)
HPAF-II	Male*	Pancreatic epithelial	Human	1982	C	(114, 158)
HT-29	Female	Colonic epithelial	Human	1964	C	(70, 271)
HuH7	Male	Hepatocyte	Human	1982	C	(171)
HuTu80	Male	Duodenal epithelial	Human	NA	C	(232)
IB3–1		Lung epithelial	Human	1992	N	(67, 297)
IEC-6	Male	Small intestine epithelial	Rat	1978	N	(208, 209)
IMR-90	Female	Lung fibroblast	Human	1977	N	(65, 176)
Jurkat	Male	Lymphoblast	Human	1984	C	(234, 276)
K562	Female	Bone marrow lymphoblast	Human	1975	C	(118, 142)
KATO III	Male*	Gastric carcinoma mixed	Human	1978	C	(238)
KG-1	Male	Macrophage	Human	1978	C	(121, 122)
LLC-PK1	Male	Kidney epithelial	Pig	1979	N	(103, 198)
LnCAP	Male	Prostate epithelial	Human	1977	C	(102)
LS 174T	Female	Colonic epithelial	Human	1976	C	(262)
Ltk-11	Male	Fibroblast	Mouse	2004	N	(124)
Mc3T3-E1		Preosteoblast	Mouse	1981	N	(273)
MCF-7	Female	Breast epithelial	Human	1970	C	(27, 250)
MDA-MB-468	Female	Breast epithelial	Human	1977	C	(192, 243)

Continued

Table 1.—Continued

Cell Line	Sex	Description	Species	Year	Origin	Reference
MDCK	Female	Kidney epithelial	Dog	1958	N	(76)
MEF-1		Fibroblast	Mouse	1993	N	(292)
MG-63	Male	Bone fibroblast	Human	1977	C	(21)
MIA-PaCa-2	Male*	Pancreatic epithelial	Human	1975	C	(293)
mIMCD-3		Kidney epithelial	Mouse	1991	N	(216)
MKN45	Female	Gastric carcinoma	Human	NA	C	(170)
NRK		Kidney epithelial	Rat	NA	N	(50)
NuLi-1	Male	Lung epithelial	Human	2000	N	(294)
OK	Female	Kidney epithelial	Opossum	1978	N	(126)
PC-3	Male*	Prostate epithelial	Human	1979	C	(42, 108)
PC12	Male	Adrenal gland	Rat	1976	N	(86)
pRSV-T	Male	Epithelial	Human	1997	N	(127)
PNT1A	Male	Prostate epithelial	Human	1991	N	(47)
RPE-1	Female	Retina pigment epithelial	Human	1998	N	(152)
Saos-2	Female	Bone epithelial	Human	1975	C	(69, 221)
SBC-3	Male	Lung carcinoma	Human	1997	C	(123, 287)
SC2G	Female	Breast epithelial	Mouse	1964	C	(163, 164)
SCC-9	Male	Tongue	Human	1981	C	(219)
SH-SY5y	Female	Bone marrow epithelial	Human	1970	C	(20, 225)
SK-MEL-2	Male	Skin melanoma	Human	1975	C	(43)
SK-MEL-24	Male	Skin melanoma	Human	1976	C	(71, 204)
SW620	Male*	Colon epithelial	Human	1976	C	(69, 136)
SW 982	Female	Synovium	Human	1974	C	(69, 288)
T84	Male*	Colonic epithelia	Human	1980	C	(12, 54, 169)
TCCSUP	Female	Urinary bladder epithelia	Human	1978	C	(87, 175)
THP-1	Male	Monocyte	Human	1982	C	(248, 264)
U-87	Male*	Glioma	Human	1966	C	(69, 205)
U-937	Male*	Monocyte	Human	1974	C	(125)
UMR-106		Bone epithelial	Rat	1976	C	(149, 191)
Y79	Female	Retinoblastoma	Human	1974	C	(151)

Where possible, references include the first descriptions of the cell lines. Cells were derived from "C," cancerous tissue or "N," noncancerous tissue (usually virally transformed). *Cells derived from human "male" tissues that now express only amelogenin-X and no amelogenin-Y. NA, original deposition date could not be determined. This table is not intended to be a comprehensive data set, but rather to highlight the cell lines that are routinely used by authors in *AJP-Cell Physiology*. For a larger database, the reader is directed to such sites as American Tissue Type Collection (ATTC.org).

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).

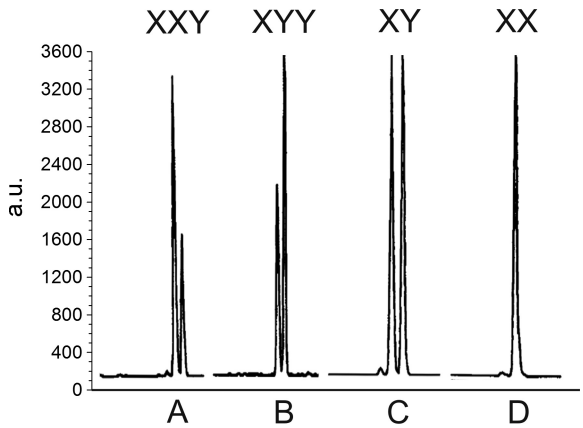

Fig. 3. Comparison of electrophoretograms of sex test PCR products generated by an ABI Gene Scanner 362A. Primers for "male" and "female" amelogenin genes were employed. A, XXY cell line DNA with 1.8:1 X:Y peak area ratio; B, XYY male with 1:1.8 X:Y peak area ratio; C, normal male DNA with 1.01:1 X:Y peak area ratio; D, normal female (note absence of Y peak). a.u., arbitrary units. [From Sullivan et al. (256) with permission.]

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 that when the same male stem cells were placed in female hosts (unaltered or ovariectomized) (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.

ACKNOWLEDGMENTS

The authors are particularly indebted to the reviewers of our manuscript for many helpful suggestions.

GRANTS

Work in the authors' laboratory is supported by Cystic Fibrosis Foundation Grant CFF BRADBU05G0 and National Institutes of Health National Heart, Lung, and Blood Institute Grant HL-102208 (to N. A. Bradbury).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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.

REFERENCES

1. **Adey A, Burton JN, Kitzman JO, Hiatt JB, Lewis AP, Martin BK, Qiu R, Lee C, Shendure J.** The haplotype-resolved genome and epigenome of the aneuploid HeLa cancer cell line. *Nature* 500: 207–211, 2013.
2. **Anderson GD.** Sex differences in drug metabolism: cytochrome P-450 and uridine diphosphate glucuronosyltransferase. *J Gend Specif Med* 5: 25–33, 2002.
3. **Anderson KE, Bishop DF, Desnick RJ.** Disorders of heme biosynthesis: X-linked sideroblastic anemias and the porphyrias. In: *The Metabolic and Molecular Basis of Inherited Disease*, edited by Scriver CR, Beaudet AL, and Sly WS. New York: McGraw-Hill, 2001, p. 2991–3062.
4. **Aristotle.** *Historia Animalium: Books VII–X*. Cambridge, MA: Harvard Univ. Press, 350 BC.
5. **Arnold AP.** Concepts of genetic and hormonal induction of vertebrate sexual differentiation in the twentieth century, with special reference to the brain. In: *Hormones, Brain and Behavior*, edited by Pfaff D, Arnold AP, Etgen AM, Fahrbach SE, and Rubin RT. New York: Academic, 2009, p. 105–135.
6. **Avery B, Jorgensen CB, Madison V, Greve T.** Morphological development and sex of bovine in vitro-fertilized embryos. *Mol Reprod Dev* 32: 265–270, 1992.
7. **Aviv A, Shay J, Christensen K, Wright W.** The longevity gender gap: are telomeres the explanation? *Sci Aging Knowledge Environ* 2005: pe16, 2005.
8. **Bancroft FC, Levine L, Tashjian AH Jr.** Control of growth hormone production by a clonal strain of rat pituitary cells. Stimulation by hydrocortisone. *J Cell Biol* 43: 432–441, 1969.
9. **Banks-Schlegel SP, Gazdar AF, Harris CC.** Intermediate filament and cross-linked envelope expression in human lung tumor cell lines. *Cancer Res* 45: 1187–1197, 1985.
10. **Barranco SC, Townsend CM Jr, Casartelli C, Macik BG, Burger NL, Boerwinkle WR, Gourley WK.** Establishment and characterization of an in vitro model system for human adenocarcinoma of the stomach. *Cancer Res* 43: 1703–1709, 1983.
11. **Barranco SC, Townsend CM Jr, Quraishi MA, Burger NL, Nevill HC, Howell KH, and Boerwinkle WR.** Heterogeneous responses of an in vitro model of human stomach cancer to anticancer drugs. *Invest New Drugs* 1: 117–127, 1983.
12. **Barrett KE, Huott PA, Shah SS, Dharmsathaphorn K, Wasserman SI.** Differing effects of apical and basolateral adenosine on colonic epithelial cell line T84. *Am J Physiol Cell Physiol* 256: C197–C203, 1989.
13. **Basson MD, Beidler DR, Turowski G, Zarif A, Modlin IM, Jena BP, Madri JA.** Effect of tyrosine kinase inhibition on basal and epidermal growth factor-stimulated human Caco-2 enterocyte sheet migration and proliferation. *J Cell Physiol* 160: 491–501, 1994.

14. **Basson MD, Modlin IM, Madri JA.** Human enterocyte (Caco-2) migration is modulated in vitro by extracellular matrix composition and epidermal growth factor. *J Clin Invest* 90: 15–23, 1992.
15. **Beery AK, Zucker I.** Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev* 35: 565–572, 2011.
16. **Beeson PB.** Age and sex associations of 40 autoimmune diseases. *Am J Med* 96: 457–462, 1994.
17. **Belmont JW.** Genetic control of X inactivation and processes leading to X-inactivation skewing. *Am J Hum Genet* 58: 1101–1108, 1996.
18. **Benda P, Lightbody J, Sato G, Levine L, Sweet W.** Differentiated rat glial cell strain in tissue culture. *Science* 161: 370–371, 1968.
19. **Bianchi I, Lleo A, Gershwin ME, Invernizzi P.** The X chromosome and immune associated genes. *J Autoimmun* 38: J187–J192, 2012.
20. **Biedler JL, Roffler-Tarlov S, Schachner M, Freedman LS.** Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. *Cancer Res* 38: 3751–3757, 1978.
21. **Billiau A, Edy VG, Heremans H, Van Damme J, Desmyter J, Georgiades JA, De Somer P.** Human interferon: mass production in a newly established cell line, MG-63. *Antimicrob Agents Chemother* 12: 11–15, 1977.
22. **Bird CM, Burgess N.** The hippocampus and memory: insights from spatial processing. *Nat Rev Neurosci* 9: 182–194, 2008.
23. **Bolin SR, Ridpath JF, Black J, Macy M, Roblin R.** Survey of cell lines in the American Type Culture Collection for bovine viral diarrhea virus. *J Virol Methods* 48: 211–221, 1994.
24. **Bolton-Maggs PH, Pasi KJ.** Haemophilias A and B. *Lancet* 361: 1801–1809, 2003.
25. **Borras C, Sastre J, Garcia-Sala D, Lloret A, Pallardo F, Vina J.** Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. *Free Radic Biol Med* 34: 546–552, 2003.
26. **Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, and zur Hausen H.** A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. *EMBO J* 3: 1151–1157, 1984.
27. **Brandes LJ, Hermonat MW.** Receptor status and subsequent sensitivity of subclones of MCF-7 human breast cancer cells surviving exposure to diethylstilbestrol. *Cancer Res* 43: 2831–2835, 1983.
28. **Brandt BL, Kimes BW, Klier FG.** Development of a clonal myogenic cell line with unusual biochemical properties. *J Cell Physiol* 88: 255–275, 1976.
29. **Brinkmann B.** Is the amelogenin sex test valid? *Int J Legal Med* 116: 63, 2002.
30. **Brower M, Carney DN, Oie HK, Gazdar AF, Minna JD.** Growth of cell lines and clinical specimens of human non-small cell lung cancer in a serum-free defined medium. *Cancer Res* 46: 798–806, 1986.
31. **Cantuti-Castelvetri I, Keller-McGandy C, Bouzou B, Asteris G, Clark TW, Frosch MP, Standaert DG.** Effects of gender on nigral gene expression and parkinson disease. *Neurobiol Dis* 26: 606–614, 2007.
32. **Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, MacLeod RA, Masters JR, Nakamura Y, Reid YA, Reddel RR, Freshney RI.** Check your cultures! A list of cross-contaminated or misidentified cell lines. *Int J Cancer* 127: 1–8, 2010.
33. **Cario E, Rosenberg IM, Brandwein SL, Beck PL, Reinecker HC, Podolsky DK.** Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. *J Immunol* 164: 966–972, 2000.
34. **Carrel L, Willard HF.** Heterogeneous gene expression from the inactive X chromosome: an X-linked gene that escapes X inactivation in some human cell lines but is inactivated in others. *Proc Natl Acad Sci USA* 96: 7364–7369, 1999.
35. **Carrel L, Willard HF.** X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature* 434: 400–404, 2005.
36. **Carruth LL, Reisert I, Arnold AP.** Sex chromosome genes directly affect brain sexual differentiation. *Nat Neurosci* 5: 933–934, 2002.
37. **Carter CL, Resnick EM, Mallampalli M, Kalbarczyk A.** Sex and gender differences in Alzheimer's disease: recommendations for future research. *J Womens Health (Larchmt)* 21: 1018–1023, 2012.
38. **Ceribelli A, Pino MS, Cecere FL.** Gender differences: implications for clinical trials and practice. *J Thorac Oncol* 2: S15–S18, 2007.
39. **Chang SC, Tucker T, Thorogood NP, Brown CJ.** Mechanisms of X-chromosome inactivation. *Front Biosci* 11: 852–866, 2006.
40. **Chao KL, Dreyfus LA.** Interaction of *Escherichia coli* heat-stable enterotoxin B with cultured human intestinal epithelial cells. *Infect Immun* 65: 3209–3217, 1997.
41. **Chatterjee R.** Cell biology. Cases of mistaken identity. *Science* 315: 928–931, 2007.
42. **Chen TR.** Chromosome identity of human prostate cancer cell lines, PC-3 and PPC-1. *Cytogenet Cell Genet* 62: 183–184, 1993.
43. **Choi HJ, Choi YH, Yee SB, Im E, Jung JH, Kim ND.** Ircinin-1 induces cell cycle arrest and apoptosis in SK-MEL-2 human melanoma cells. *Mol Carcinog* 44: 162–173, 2005.
44. **Christensen J, El-Gebali S, Natoli M, Sengstag T, Delorenzi M, Bentz S, Bouzourene H, Rumbo M, Felsani A, Siissalo S, Hirvonen J, Vila MR, Saletti P, Aguet M, Anderle P.** Defining new criteria for selection of cell-based intestinal models using publicly available databases. *BMC Genomics* 13: 274, 2012.
45. **Conley ME, Brown P, Pickard AR, Buckley RH, Miller DS, Raskind WH, Singer JW, Fialkow PJ.** Expression of the gene defect in X-linked agammaglobulinemia. *N Engl J Med* 315: 564–567, 1986.
46. **Cozens AL, Yezzi MJ, Kunzelmann K, Ohrui T, Chin L, Eng K, Finkbeiner WE, Widdicombe JH, Gruenert DC.** CFTR expression and chloride secretion in polarized immortal human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 10: 38–47, 1994.
47. **Cussenot O, Berthon P, Berger R, Mowszowicz I, Faille A, Hojman F, Teillac P, Le Duc A, Calvo F.** Immortalization of human adult normal prostatic epithelial cells by liposomes containing large T-SV40 gene. *J Urol* 146: 881–886, 1991.
48. **Darlington GJ.** Liver cell lines. *Methods Enzymol* 151: 19–38, 1987.
49. **Darlington GJ, Bernhard HP, Miller RA, Ruddle FH.** Expression of liver phenotypes in cultured mouse hepatoma cells. *J Natl Cancer Inst* 64: 809–819, 1980.
50. **de Larco JE, Todaro GJ.** Epithelioid and fibroblastic rat kidney cell clones: epidermal growth factor (EGF) receptors and the effect of mouse sarcoma virus transformation. *J Cell Physiol* 94: 335–342, 1978.
51. **Deasy BM, Lu A, Tebbets JC, Feduska JM, Schugar RC, Pollett JB, Sun B, Urish KL, Gharaibeh BM, Cao B, Rubin RT, Huard J.** A role for cell sex in stem cell-mediated skeletal muscle regeneration: female cells have higher muscle regeneration efficiency. *J Cell Biol* 177: 73–86, 2007.
52. **Demko CA, Byard PJ, Davis PB.** Gender differences in cystic fibrosis: *Pseudomonas aeruginosa* infection. *J Clin Epidemiol* 48: 1041–1049, 1995.
53. **Dewing P, Shi T, Horvath S, Vilain E.** Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. *Brain Res Mol Brain Res* 118: 82–90, 2003.
54. **Dharmsathaphorn K, McRoberts JA, Mandel KG, Tisdale LD, Masui H.** A human colonic tumor cell line that maintains vectorial electrolyte transport. *Am J Physiol Gastrointest Liver Physiol* 246: G204–G208, 1984.
55. **Disteche CM.** The great escape. *Am J Hum Genet* 60: 1312–1315, 1997.
56. **Doetschman T, Maeda N, Smithies O.** Targeted mutation of the Hprt gene in mouse embryonic stem cells. *Proc Natl Acad Sci USA* 85: 8583–8587, 1988.
57. **Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R.** The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. *J Embryol Exp Morphol* 87: 27–45, 1985.
58. **Donders J, Hoffman NM.** Gender differences in learning and memory after pediatric traumatic brain injury. *Neuropsychology* 16: 491–499, 2002.
59. **Dronamraju KR.** Y-linkage in man. *Nature* 201: 424–425, 1964.
60. **Du L, Bayir H, Lai Y, Zhang X, Kochanek PM, Watkins SC, Graham SH, Clark RS.** Innate gender-based proclivity in response to cytotoxicity and programmed cell death pathway. *J Biol Chem* 279: 38563–38570, 2004.
61. **Dumenco L, Oguey D, Wu J, Messier N, Fausto N.** Introduction of a murine p53 mutation corresponding to human codon 249 into a murine hepatocyte cell line results in growth advantage, but not in transformation. *Hepatology* 22: 1279–1288, 1995.
62. **Dunn KC, Aotaki-Keen AE, Putkey FR, Hjelmeland LM.** ARPE-19, a human retinal pigment epithelial cell line with differentiated properties. *Exp Eye Res* 62: 155–169, 1996.
63. **Durkin AS, Cedrone E, Sykes G, Boles D, Reid YA.** Utility of gender determination in cell line identity. *In Vitro Cell Dev Biol Anim* 36: 344–347, 2000.

64. **Eastoe JE.** The chemical composition of bone and tooth. *Adv Fluorine Res* 21: 5–17, 1965.
65. **Ehler E, Babiychuk E, Draeger A.** Human foetal lung (IMR-90) cells: myofibroblasts with smooth muscle-like contractile properties. *Cell Motil Cytoskeleton* 34: 288–298, 1996.
66. **Fisher EM, Beer-Romero P, Brown LG, Ridley A, McNeil JA, Lawrence JB, Willard HF, Bieber FR, Page DC.** Homologous ribosomal protein genes on the human X and Y chromosomes: escape from X inactivation and possible implications for Turner syndrome. *Cell* 63: 1205–1218, 1990.
67. **Flotte TR, Afione SA, Solow R, Drumm ML, Markakis D, Guggino WB, Zeitlin PL, Carter BJ.** Expression of the cystic fibrosis transmembrane conductance regulator from a novel adeno-associated virus promoter. *J Biol Chem* 268: 3781–3790, 1993.
68. **Fogh J.** Cultivation, characterization, and identification of human tumor cells with emphasis on kidney, testis, and bladder tumors. *Natl Cancer Inst Monogr*: 5–9, 1978.
69. **Fogh J, Fogh JM, Orfeo T.** One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. *J Natl Cancer Inst* 59: 221–226, 1977.
70. **Fogh J, Trempe G.** New human tumor cell lines. In: *Human Tumor Cells In Vitro*, edited by Fogh J. New York: Plenum, 1975, p. 115–159.
71. **Fogh J, Wright WC, Loveless JD.** Absence of HeLa cell contamination in 169 cell lines derived from human tumors. *J Natl Cancer Inst* 58: 209–214, 1977.
72. **Ford CE, Jones KW, Polani PE, De Almeida JC, Briggs JH.** A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet* 1: 711–713, 1959.
73. **Franco R, Cidlowski JA.** Glutathione efflux and cell death. *Antioxid Redox Signal* 17: 1694–1713, 2012.
74. **Freije D, Helms C, Watson MS, and Donis-Keller H.** Identification of a second pseudoautosomal region near the Xq and Yq telomeres. *Science* 258: 1784–1787, 1992.
74a. **Fuller CM, Insel PA.** *I don't know the question, but sex is definitely the answer!* Focus on "In pursuit of scientific excellence: sex matters." *Am J Physiol Cell Physiol* 302: C1269–C1270, 2012; and "Do you know the sex of your cells?" *Am J Physiol Cell Physiol* (November 6, 2013), doi:10.1152/ajpcell.00281.2013.
75. **Gallagher R, Collins S, Trujillo J, McCredie K, Ahearn M, Tsai S, Metzgar R, Aulakh G, Ting R, Ruscetti F, Gallo R.** Characterization of the continuous, differentiating myeloid cell line (HL-60) from a patient with acute promyelocytic leukemia. *Blood* 54: 713–733, 1979.
76. **Gaush CR, Hard WL, Smith TF.** Characterization of an established line of canine kidney cells (MDCK). *Proc Soc Exp Biol Med* 122: 931–935, 1966.
77. **Gazdar AF, Linnoila RI, Kurita Y, Oie HK, Mulshine JL, Clark JC, Whitsett JA.** Peripheral airway cell differentiation in human lung cancer cell lines. *Cancer Res* 50: 5481–5487, 1990.
78. **Gey GO, Coffman WD, Kubicek MT.** Tissue culture studies on the proliferative capacity of cervical carcinoma and normal epithelium. *Cancer Res* 12: 264–265, 1952.
79. **Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP.** In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. *J Natl Cancer Inst* 51: 1417–1423, 1973.
80. **Ginsberg HS, Young CHS.** Genetics of adenoviruses. In: *Comprehensive Virology*, edited by Fraenkel-Conrat RR and Wagner H. New York: Plenum, 1977, p. 27–88.
81. **Gluzman Y.** SV40-transformed simian cells support the replication of early SV40 mutants. *Cell* 23: 175–182, 1981.
82. **Gompel A, Piette JC.** Systemic lupus erythematosus and hormone replacement therapy. *Menopause Int* 13: 65–70, 2007.
83. **Graham FL, Smiley J, Russell WC, Nairn R.** Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol* 36: 59–74, 1977.
84. **Green H. (Inventor)** Triglyceride- accumulating clonal cell line. US patent 4,003,789, January 18, 1977.
85. **Green H, Meuth M.** An established pre-adipose cell line and its differentiation in culture. *Cell* 3: 127–133, 1974.
86. **Greene LA, Tischler AS.** Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc Natl Acad Sci USA* 73: 2424–2428, 1976.
87. **Grimm MO, Jurgens B, Schulz WA, Decken K, Makri D, Schmitz-Drager BJ.** Inactivation of tumor suppressor genes and deregulation of the c-myc gene in urothelial cancer cell lines. *Urol Res* 23: 293–300, 1995.
88. **Gruenert DC, Finkbeiner WE, Widdicombe JH.** Culture and transformation of human airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 268: L347–L360, 1995.
89. **Grumbach MM, Hughes IA, Conte FA.** Disorders of sex differentiation. In: *Textbook of Endocrinology* (10 ed.), edited by Larsen PR, Kronenberg HM, Melmed S, and Polansky S. Philadelphia: Saunders (Elsevier), 2003, p. 865.
90. **Guengerich FP.** Human cytochrome P450 enzymes. In: *Cytochrome P450: Structure, Mechanism, and Biochemistry*, edited by Ortiz de Montellano PR. New York: Plenum, 1995, p. 473–535.
91. **Haas-Rochholz H, Weiler G.** Additional primer sets for an amelogenin gene PCR-based DNA-sex test. *Int J Legal Med* 110: 312–315, 1997.
92. **Haaxma CA, Bloem BR, Borm GF, Oyen WJ, Leenders KL, Eshuis S, Booij J, Dluzen DE, Horstink MW.** Gender differences in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 78: 819–824, 2007.
93. **Haggerty DF, Young PL, Popjak G, Carnes WH.** Phenylalanine hydroxylase in cultured hepatoxytes. I. Hormonal control of enzyme levels. *J Biol Chem* 248: 223–232, 1973.
94. **Hanauske U, Hanauske AR, Clark GM, Tsen D, Buchok J, von Hoff DD.** A new in vitro screening system for anticancer drugs for the treatment of non-small cell lung cancer. *Sel Cancer Ther* 5: 97–111, 1989.
95. **Harris SA, Enger RJ, Riggs BL, Spelsberg TC.** Development and characterization of a conditionally immortalized human fetal osteoblastic cell line. *J Bone Miner Res* 10: 178–186, 1995.
96. **Harris SA, Spelsberg TC. (Inventors)** Immortalized human fetal osteoblastic cells. US patent 5,681,701, October 28, 1997.
97. **Heard E, Disteche CM.** Dosage compensation in mammals: fine-tuning the expression of the X chromosome. *Genes Dev* 20: 1848–1867, 2006.
98. **Henke A, Fischer C, Rappold GA.** Genetic map of the human pseudoautosomal region reveals a high rate of recombination in female meiosis at the Xp telomere. *Genomics* 18: 478–485, 1993.
99. **Heyer A, Hasselblatt M, von Ahsen N, Hafner H, Siren AL, Ehrenreich H.** In vitro gender differences in neuronal survival on hypoxia and in 17beta-estradiol-mediated neuroprotection. *J Cereb Blood Flow Metab* 25: 427–430, 2005.
100. **Hiort O, Holterhus PM.** The molecular basis of male sexual differentiation. *Eur J Endocrinol* 142: 101–110, 2000.
101. **Holtkamp GM, Van Rossem M, de Vos AF, Willekens B, Peek R, Kijlstra A.** Polarized secretion of IL-6 and IL-8 by human retinal pigment epithelial cells. *Clin Exp Immunol* 112: 34–43, 1998.
102. **Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Chu TM, Mirand EA, Murphy GP.** LNCaP model of human prostatic carcinoma. *Cancer Res* 43: 1809–1818, 1983.
103. **Hull RN, Cherry WR, Weaver GW.** The origin and characteristics of a pig kidney cell strain, LLC-PK. *In Vitro* 12: 670–677, 1976.
104. **Jacobs PA, Strong JA.** A case of human intersexuality having a possible XXY sex-determining mechanism. *Nature* 183: 302–303, 1959.
105. **Jensen FC, Girardi AJ, Gilden RV, Koprowski H.** Infection of human and simian tissue cultures with Rous sarcoma virus. *Proc Natl Acad Sci USA* 52: 53–59, 1964.
106. **Jones HW Jr, McKusick VA, Harper PS, Wuu KD.** George Otto Gey. (1899–1970). The HeLa cell and a reappraisal of its origin. *Obstet Gynecol* 38: 945–949, 1971.
107. **Jumarie C, Malo C.** Caco-2 cells cultured in serum-free medium as a model for the study of enterocytic differentiation in vitro. *J Cell Physiol* 149: 24–33, 1991.
108. **Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW.** Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Invest Urol* 17: 16–23, 1979.
109. **Kalyanaraman B.** Teaching the basics of redox biology to medical and graduate students: oxidants, antioxidants and disease mechanisms. *Redox Biol* 1: 244–257, 2013.
110. **Kao FT, Puck TT.** Genetics of somatic mammalian cells. IV. Properties of Chinese hamster cell mutants with respect to the requirement for proline. *Genetics* 55: 513–524, 1967.
111. **Kashimada K, Koopman P.** Sry: the master switch in mammalian sex determination. *Development* 137: 3921–3930, 2010.
112. **Katayose Y, Kim M, Rakkar AN, Li Z, Cowan KH, Seth P.** Promoting apoptosis: a novel activity associated with the cyclin-dependent kinase inhibitor p27. *Cancer Res* 57: 5441–5445, 1997.

113. **Kent-First M, Muallem A, Shultz J, Pryor J, Roberts K, Nolten W, Meisner L, Chandley A, Gouchy G, Jorgensen L, Havighurst T, Grosch J.** Defining regions of the Y-chromosome responsible for male infertility and identification of a fourth AZF region (AZFd) by Y-chromosome microdeletion detection. *Mol Reprod Dev* 53: 27–41, 1999.
114. **Kim YW, Kern HF, Mullins TD, Koriwchak MJ, Metzgar RS.** Characterization of clones of a human pancreatic adenocarcinoma cell line representing different stages of differentiation. *Pancreas* 4: 353–362, 1989.
115. **Kimes BW, Brandt BL.** Characterization of two putative smooth muscle cell lines from rat thoracic aorta. *Exp Cell Res* 98: 349–366, 1976.
116. **Kimes BW, Brandt BL.** Properties of a clonal muscle cell line from rat heart. *Exp Cell Res* 98: 367–381, 1976.
117. **Kit S, Dubbs DR, DeTorres RA, Melnick JL.** Enhanced thymidine kinase activity following infection of green monkey kidney cells by simian adenoviruses, simian papovavirus SV40, and an adenovirus-SV40 "hybrid". *Virology* 27: 453–457, 1965.
118. **Klein E, Ben-Bassat H, Neumann H, Ralph P, Zeuthen J, Polliack A, Vanky F.** Properties of the K562 cell line, derived from a patient with chronic myeloid leukemia. *Int J Cancer* 18: 421–431, 1976.
119. **Knowles BB, Aden DP.** Human hepatoma derived cell line, process for preparation thereof, and uses therefor. US patent 4,393,133, July 12, 1983.
120. **Knowles BB, Howe CC, Aden DP.** Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science* 209: 497–499, 1980.
121. **Koeffler HP, Golde DW.** Acute myelogenous leukemia: a human cell line responsive to colony-stimulating activity. *Science* 200: 1153–1154, 1978.
122. **Koeffler HP, Golde DW.** Human myeloid leukemia cell lines: a review. *Blood* 56: 344–350, 1980.
123. **Kohara H, Tabata M, Kiura K, Ueoka H, Kawata K, Chikamori M, Aoe K, Chikamori K, Matsushita A, Harada M.** Synergistic effects of topoisomerase I inhibitor, 7-ethyl-10-hydroxycamptothecin, and irradiation in a cisplatin-resistant human small cell lung cancer cell line. *Clin Cancer Res* 8: 287–292, 2002.
124. **Konkel M.** Compounds specific for the human. alpha.1d adrenergic receptor and uses thereof. US patent 6,706,716, March 16, 2004.
125. **Koren HS, Anderson SJ, Larrick JW.** In vitro activation of a human macrophage-like cell line. *Nature* 279: 328–331, 1979.
126. **Koyama H, Goodpasture C, Miller MM, Teplitz RL, Riggs AD.** Establishment and characterization of a cell line from the American opossum (*Didelphys virginiana*). *In Vitro* 14: 239–246, 1978.
127. **Kruszewski FH, Walker TL, DiPasquale LC.** Evaluation of a human corneal epithelial cell line as an in vitro model for assessing ocular irritation. *Fundam Appl Toxicol* 36: 130–140, 1997.
128. **Kuroda R, Usas A, Kubo S, Corsi K, Peng H, Rose T, Cummins J, Fu FH, Huard J.** Cartilage repair using bone morphogenetic protein 4 and muscle-derived stem cells. *Arthritis Rheum* 54: 433–442, 2006.
129. **Lahn BT, Page DC.** Functional coherence of the human Y chromosome. *Science* 278: 675–680, 1997.
130. **Landry JJ, Pyl PT, Rausch T, Zichner T, Tekkedil MM, Stutz AM, Jauch A, Aiyar RS, Pau G, Delhomme N, Gagneur J, Korbel JO, Huber W, Steinmetz LM.** The genomic and transcriptomic landscape of a HeLa Cell line. *G3 (Bethesda)* 3: 1213–1224, 2013.
131. **Lange B, Valtieri M, Santoli D, Caracciolo D, Mavilio F, Gemperlein I, Griffin C, Emanuel B, Finan J, Nowell P, Rovera G.** Growth factor requirements of childhood acute leukemia: establishment of GM-CSF-dependent cell lines. *Blood* 70: 192–199, 1987.
132. **Lau EC, Mohandas TK, Shapiro LJ, Slavkin HC, Snead ML.** Human and mouse amelogenin gene loci are on the sex chromosomes. *Genomics* 4: 162–168, 1989.
133. **Lavenex P, Banta Lavenex P.** Building hippocampal circuits to learn and remember: insights into the development of human memory. *Behav Brain Res* 254: 8–21, 2013.
134. **Lees AJ, Hardy J, Revesz T.** Parkinson's disease. *Lancet* 373: 2055–2066, 2009.
135. **Leggio GM, Salomone S, Bucolo C, Platania C, Micale V, Caraci F, Drago F.** Dopamine D_3 receptor as a new pharmacological target for the treatment of depression. *Eur J Pharmacol*. doi:10.1016/j.ejphar.2013.07.022. [Epub ahead of print]
136. **Lelbovitz A, Wright WC, Pathak S, Siciliano MJ, Daniels WP.** Detection and analysis of a glucose 6-phosphate dehydrogenase phenotype B cell line contamination. *J Natl Cancer Inst* 63: 635–645, 1979.
137. **Lieber M, Smith B, Szakal A, Nelson-Rees W, Todaro G.** A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. *Int J Cancer* 17: 62–70, 1976.
138. **Lightbody J.** Establishment of differentiated clonal strains of glial brain cells in culture (Abstract). *Fed Proc* 27: 720, 1968.
139. **Littlewood-Evans AJ, Bilbe G, Bowler WB, Farley D, Wlodarski B, Kokubo T, Inaoka T, Sloane J, Evans DB, Gallagher JA.** The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. *Cancer Res* 57: 5386–5390, 1997.
140. **Lockshin MD.** Invited review: sex ratio and rheumatic disease. *J Appl Physiol* 91: 2366–2373, 2001.
141. **Lowy DR, Rands E, Scolnick EM.** Helper-independent transformation by unintegrated Harvey sarcoma virus DNA. *J Virol* 26: 291–298, 1978.
142. **Lozzio CB, Lozzio BB.** Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. *Blood* 45: 321–334, 1975.
143. **Lyon MF.** Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature* 190: 372–373, 1961.
144. **Lyons KE, Hubble JP, Troster AI, Pahwa R, Koller WC.** Gender differences in Parkinson's disease. *Clin Neuropharmacol* 21: 118–121, 1998.
145. **MacLusky NJ, Naftolin F.** Sexual differentiation of the central nervous system. *Science* 211: 1294–1302, 1981.
146. **Macpherson I, Stoker M.** Polyoma transformation of hamster cell clones–an investigation of genetic factors affecting cell competence. *Virology* 16: 147–151, 1962.
147. **Mannucci A, Sullivan KM, Ivanov PL, Gill P.** Forensic application of a rapid and quantitative DNA sex test by amplification of the X-Y homologous gene amelogenin. *Int J Legal Med* 106: 190–193, 1994.
148. **Mapherson I.** Characteristics of a hamster cell clone transformed by polyoma virus. *J Natl Cancer Inst* 30: 795–815, 1963.
149. **Martin TJ, Ingleton PM, Underwood JC, Michelangeli VP, Hunt NH, Melick RA.** Parathyroid hormone-responsive adenylate cyclase in induced transplantable osteogenic rat sarcoma. *Nature* 260: 436–438, 1976.
150. **Masters JR.** HeLa cells 50 years on: the good, the bad and the ugly. *Nat Rev Cancer* 2: 315–319, 2002.
151. **Matsuki Y, Nakashima M, Amizuka N, Warshawsky H, Goltzman D, Yamada KM, Yamada Y.** A compilation of partial sequences of randomly selected cDNA clones from the rat incisor. *J Dent Res* 74: 307–312, 1995.
152. **Matsunaga H, Handa JT, Aotaki-Keen A, Sherwood SW, West MD, Hjelmeland LM.** Beta-galactosidase histochemistry and telomere loss in senescent retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 40: 197–202, 1999.
153. **McAllister RM, Gardner MB, Greene AE, Bradt C, Nichols WW, Landing BH.** Cultivation in vitro of cells derived from a human osteosarcoma. *Cancer* 27: 397–402, 1971.
154. **McDonough PG.** The Y-chromosome and reproductive disorders. *Reprod Fertil Dev* 10: 1–16, 1998.
155. **McIntosh JC, Schoumacher RA, Tiller RE.** Pancreatic adenocarcinoma in a patient with cystic fibrosis. *Am J Med* 85: 592, 1988.
156. **McKusick-Nathans Institute of Genetic Medicine.** *Online Mendelian Inheritance in Man*. Baltimore, MD: Johns Hopkins University. http://omim.org/.
157. **Meszaros LB, Usas A, Cooper GM, Huard J.** Effect of host sex and sex hormones on muscle-derived stem cell-mediated bone formation and defect healing. *Tissue Eng Part A* 18: 1751–1759, 2012.
158. **Metzgar RS, Gaillard MT, Levine SJ, Tuck FL, Bossen EH, Borowitz MJ.** Antigens of human pancreatic adenocarcinoma cells defined by murine monoclonal antibodies. *Cancer Res* 42: 601–608, 1982.
159. **Meyer-Lindenberg A, Miletich RS, Kohn PD, Esposito G, Carson RE, Quarantelli M, Weinberger DR, Berman KF.** Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia. *Nat Neurosci* 5: 267–271, 2002.
160. **Michaelides M, Hardcastle AJ, Hunt DM, Moore AT.** Progressive cone and cone-rod dystrophies: phenotypes and underlying molecular genetic basis. *Surv Ophthalmol* 51: 232–258, 2006.
161. **Miller VM.** In pursuit of scientific excellence: sex matters. *Adv Physiol Educ* 36: 83–84, 2012.
162. **Milo GE, Shuler CF, Stoner G, Chen JC.** Conversion of premalignant human cells to tumorigenic cells by methylmethane sulfonate and methylnitronitrosoguanidine. *Cell Biol Toxicol* 8: 193–205, 1992.
163. **Minesita T, Yamaguchi K.** An androgen-dependent mouse mammary tumor. *Cancer Res* 25: 1168–1175, 1965.

164. **Minesita T, Yamaguchi K.** An androgen-dependent tumor derived from a hormone- independent spontaneous tumor of a female mouse. *Steroids* 4: 815–830, 1964.
165. **Mizell M.** *Biology of Amphibian Tumors*. New York: Springer-Verlag, 1969.
166. **Mollerup S, Ryberg D, Hewer A, Phillips DH, Haugen A.** Sex differences in lung CYP1A1 expression and DNA adduct levels among lung cancer patients. *Cancer Res* 59: 3317–3320, 1999.
167. **Muller HJ.** Further studies on the nature and causes of gene mutation. *Proc 6th Int Congr Genet Ithaca, New York*, 1932, p. 213–255.
168. **Muntoni F, Torelli S, Ferlini A.** Dystrophin and mutations: one gene, several proteins, multiple phenotypes. *Lancet Neurol* 2: 731–740, 2003.
169. **Murakami H, Masui H.** Hormonal control of human colon carcinoma cell growth in serum-free medium. *Proc Natl Acad Sci USA* 77: 3464–3468, 1980.
170. **Naito Y, Kino I, Horiuchi K, Fujimoto D.** Promotion of collagen production by human fibroblasts with gastric cancer cells in vitro. *Virchows Arch B Cell Pathol Incl Mol Pathol* 46: 145–154, 1984.
171. **Nakabayashi H, Taketa K, Miyano K, Yamane T, Sato J.** Growth of human hepatoma cells lines with differentiated functions in chemically defined medium. *Cancer Res* 42: 3858–3863, 1982.
172. **Nakahori Y, Takenaka O, Nakagome Y.** A human X-Y homologous region encodes "amelogenin". *Genomics* 9: 264–269, 1991.
173. **Nardone RM.** Eradication of cross-contaminated cell lines: a call for action. *Cell Biol Toxicol* 23: 367–372, 2007.
174. **Nathans J, Piantanida TP, Eddy RL, Shows TB, Hogness DS.** Molecular genetics of inherited variation in human color vision. *Science* 232: 203–210, 1986.
175. **Nayak SK, O'Toole C, Price ZH.** A cell line from an anaplastic transitional cell carcinoma of human urinary bladder. *Br J Cancer* 35: 142–151, 1977.
176. **Nichols WW, Murphy DG, Cristofalo VJ, Toji LH, Greene AE, Dwight SA.** Characterization of a new human diploid cell strain, IMR-90. *Science* 196: 60–63, 1977.
177. **Nieuwenhoven L, Klinge I.** Scientific excellence in applying sex- and gender-sensitive methods in biomedical and health research. *J Womens Health (Larchmt)* 19: 313–321, 2010.
178. **Norris JS, Cornett LE, Hardin JW, Kohler PO, MacLeod SL, Srivastava A, Syms AJ, Smith RG.** Autocrine regulation of growth: II. Glucocorticoids inhibit transcription of c-$_{sis}$ oncogene-specific RNA transcripts. *Biochem Biophys Res Commun* 122: 124–128, 1984.
179. **O'Reilly MA, Gazdar AF, Morris RE, Whitsett JA.** Differential effects of glucocorticoid on expression of surfactant proteins in a human lung adenocarcinoma cell line. *Biochim Biophys Acta* 970: 194–204, 1988.
180. **Ohno S.** *Sex Chromosomes and Sex-Linked Genes*. New York: Springer-Verlag, 1967.
181. **Osgood MP.** X-chromosome inactivation: the case of the calico cat. *Am J Pharm Educ* 58: 204–205, 1994.
182. **Ostrer H.** Sex-based differences in gene transmission and gene expression. *Lupus* 8: 365–369, 1999.
183. **Owen NE.** Effect of TPA on ion fluxes and DNA synthesis in vascular smooth muscle cells. *J Cell Biol* 101: 454–459, 1985.
184. **Packard MG, Knowlton BJ.** Learning and memory functions of the Basal Ganglia. *Annu Rev Neurosci* 25: 563–593, 2002.
185. **Page DC, Disteche CM, Simpson EM, de la Chapelle A, Andersson M, Alitalo T, Brown LG, Green P, Akots G.** Chromosomal localization of ZFX–a human gene that escapes X inactivation–and its murine homologs. *Genomics* 7: 37–46, 1990.
186. **Paine MF, Ludington SS, Chen ML, Stewart PW, Huang SM, Watkins PB.** Do men and women differ in proximal small intestinal CYP3A or P-glycoprotein expression? *Drug Metab Dispos* 33: 426–433, 2005.
187. **Papsidero LD, Kuriyama M, Wang MC, Horoszewicz J, Leong SS, Valenzuela L, Murphy GP, Chu TM.** Prostate antigen: a marker for human prostate epithelial cells. *J Natl Cancer Inst* 66: 37–42, 1981.
188. **Park JG, Frucht H, LaRocca RV, Bliss DP Jr, Kurita Y, Chen TR, Henslee JG, Trepel JB, Jensen RT, Johnson BE, Bang YJ, Kim JP, Gazdar AF.** Characteristics of cell lines established from human gastric carcinoma. *Cancer Res* 50: 2773–2780, 1990.
189. **Park JG, Lee JH, Kang MS, Park KJ, Jeon YM, Lee HJ, Kwon HS, Park HS, Yeo KS, Lee KU, Kim ST, Chung JK, Hwang YJ, Lee HS, Kim CY, Lee YI, Chen TR, Hay RJ, Song SY, Kim WH, Kim YI.** Characterization of cell lines established from human hepatocellular carcinoma. *Int J Cancer* 62: 276–282, 1995.
190. **Park SJ, Jeong SY, Kim HJ.** Y chromosome loss and other genomic alterations in hepatocellular carcinoma cell lines analyzed by CGH and CGH array. *Cancer Genet Cytogenet* 166: 56–64, 2006.
191. **Partridge NC, Frampton RJ, Eisman JA, Michelangeli VP, Elms E, Bradley TR, Martin TJ.** Receptors for 1,25(OH)2-vitamin D3 enriched in cloned osteoblast-like rat osteogenic sarcoma cells. *FEBS Lett* 115: 139–142, 1980.
192. **Pathak S, Siciliano MJ, Cailleau R, Wiseman CL, Hsu TC.** A human breast adenocarcinoma with chromosome and isoenzyme markers similar to those of the HeLa line. *J Natl Cancer Inst* 62: 263–271, 1979.
193. **Pattillo RA, Gey GO.** The establishment of a cell line of human hormone-synthesizing trophoblastic cells in vitro. *Cancer Res* 28: 1231–1236, 1968.
194. **Pattillo RA, Gey GO, Delfs E, Mattingly RF.** Human hormone production in vitro. *Science* 159: 1467–1469, 1968.
195. **Pattillo RA, Hussa RO, Story MT, Ruckert AC, Shalaby MR, Mattingly RF.** Tumor antigen and human chorionic gonadotropin in CaSki cells: a new epidermoid cervical cancer cell line. *Science* 196: 1456–1458, 1977.
196. **Payer B, Lee JT.** X chromosome dosage compensation: how mammals keep the balance. *Annu Rev Genet* 42: 733–772, 2008.
197. **Penaloza C, Estevez B, Orlanski S, Sikorska M, Walker R, Smith C, Smith B, Lockshin RA, Zakeri Z.** Sex of the cell dictates its response: differential gene expression and sensitivity to cell death inducing stress in male and female cells. *FASEB J* 23: 1869–1879, 2009.
198. **Perantoni A, Berman JJ.** Properties of Wilms' tumor line (TuWi) and pig kidney line (LLC-PK1) typical of normal kidney tubular epithelium. *In Vitro* 15: 446–454, 1979.
199. **Pergament E, Fiddler M, Cho N, Johnson D, Holmgren WJ.** Sexual differentiation and preimplantation cell growth. *Hum Reprod* 9: 1730–1732, 1994.
200. **Peterson MD, Mooseker MS.** Characterization of the enterocyte-like brush border cytoskeleton of the C2BBe clones of the human intestinal cell line, Caco-2. *J Cell Sci* 102: 581–600, 1992.
201. **Peterson WD Jr, Stulberg CS, Swanborg NK, Robinson AR.** Glucose-6-phosphate dehydrogenase isoenzymes in human cell cultures determined by sucrose-agar gel and cellulose acetate zymograms. *Proc Soc Exp Biol Med* 128: 772–776, 1968.
202. **Pilgrim C, Reisert I.** Differences between male and female brains–developmental mechanisms and implications. *Horm Metab Res* 24: 353–359, 1992.
203. **Pitot HC, Peraino C, Morse PA Jr, Potter VR.** Hepatomas in tissue culture compared with adapting liver in vivo. *Natl Cancer Inst Monogr* 13: 229–245, 1964.
204. **Pollack MS, Heagney SD, Livingston PO, Fogh J.** HLA-A, B, C and DR alloantigen expression on forty-six cultured human tumor cell lines. *J Natl Cancer Inst* 66: 1003–1012, 1981.
205. **Ponten J, Macintyre EH.** Long term culture of normal and neoplastic human glia. *Acta Pathol Microbiol Scand* 74: 465–486, 1968.
206. **Puck JM, Willard HF.** X inactivation in females with X-linked disease. *N Engl J Med* 338: 325–328, 1998.
207. **Puck TT, Cieciura SJ, Robinson A.** Genetics of somatic mammalian cells. III. Long-term cultivation of euploid cells from human and animal subjects. *J Exp Med* 108: 945–956, 1958.
208. **Quaroni A, Isselbacher KJ, Ruoslahti E.** Fibronectin synthesis by epithelial crypt cells of rat small intestine. *Proc Natl Acad Sci USA* 75: 5548–5552, 1978.
209. **Quaroni A, Wands J, Trelstad RL, Isselbacher KJ.** Epithelioid cell cultures from rat small intestine. Characterization by morphologic and immunologic criteria. *J Cell Biol* 80: 248–265, 1979.
210. **Quintana-Murci L, Fellous M.** The human Y chromosome: the biological role of a "functional wasteland". *J Biomed Biotechnol* 1: 18–24, 2001.
211. **Raccor BS, Kaspera R.** Extra-hepatic isozymes from the CYP1 and CYP2 families as potential chemotherapeutic targets. *Curr Top Med Chem* 13: 1441–1453, 2013.
212. **Ralph P.** Retention of lymphocyte characteristics by myelomas and θ^+-lymphomas: sensitivity to cortisol and phytohemagglutinin. *J Immunol* 110: 1470–1475, 1973.
213. **Ralph P, Nakoinz I.** Inhibitory effects of lectins and lymphocyte mitogens on murine lymphomas and myelomas. *J Natl Cancer Inst* 51: 883–890, 1973.

214. **Rapp F, Hsu TC.** Viruses and mammalian chromosomes. IV. Replication of herpes simplex virus in diploid Chinese hamster cells. *Virology* 25: 401–411, 1965.
215. **Rappold GA.** The pseudoautosomal regions of the human sex chromosomes. *Hum Genet* 92: 315–324, 1993.
216. **Rauchman MI, Nigam SK, Delpire E, Gullans SR.** An osmotically tolerant inner medullary collecting duct cell line from an SV40 transgenic mouse. *Am J Physiol Renal Fluid Electrolyte Physiol* 265: F416–F424, 1993.
217. **Reisert I, Engele J, Pilgrim C.** Early sexual differentiation of diencephalic dopaminergic neurons of the rat in vitro. *Cell Tissue Res* 255: 411–417, 1989.
218. **Reisert I, Pilgrim C.** Sexual differentiation of monoaminergic neurons–genetic or epigenetic? *Trends Neurosci* 14: 468–473, 1991.
219. **Rheinwald JG, Beckett MA.** Tumorigenic keratinocyte lines requiring anchorage and fibroblast support cultured from human squamous cell carcinomas. *Cancer Res* 41: 1657–1663, 1981.
220. **Robinson TE, Berridge KC.** The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev* 18: 247–291, 1993.
221. **Rodan SB, Imai Y, Thiede MA, Wesolowski G, Thompson D, Bar-Shavit Z, Shull S, Mann K, Rodan GA.** Characterization of a human osteosarcoma cell line (Saos-2) with osteoblastic properties. *Cancer Res* 47: 4961–4966, 1987.
222. **Rokaw MD, Benos DJ, Palevsky PM, Cunningham SA, West ME, Johnson JP.** Regulation of a sodium channel-associated G-protein by aldosterone. *J Biol Chem* 271: 4491–4496, 1996.
223. **Rosenfeld M, Davis R, FitzSimmons S, Pepe M, Ramsey B.** Gender gap in cystic fibrosis mortality. *Am J Epidemiol* 145: 794–803, 1997.
224. **Ross MT, Grafham DV, Coffey AJ, Scherer S, McLay K, Muzny D, Platzer M, Howell GR, Burrows C, Bird CP, Frankish A, Lovell FL, Howe KL, Ashurst JL, Fulton RS, Sudbrak R, Wen G, Jones MC, Hurles ME, Andrews TD, Scott CE, Searle S, Ramser J, Whittaker A, Deadman R, Carter NP, Hunt SE, Chen R, Cree A, Gunaratne P, Havlak P, Hodgson A, Metzker ML, Richards S, Scott G, Steffen D, Sodergren E, Wheeler DA, Worley KC, Ainscough R, Ambrose KD, Ansari-Lari MA, Aradhya S, Ashwell RI, Babbage AK, Bagguley CL, Ballabio A, Banerjee R, Barker GE, Barlow KF, Barrett IP, Bates KN, Beare DM, Beasley H, Beasley O, Beck A, Bethel G, Blechschmidt K, Brady N, Bray-Allen S, Bridgeman AM, Brown AJ, Brown MJ, Bonnin D, Bruford EA, Buhay C, Burch P, Burford D, Burgess J, Burrill W, Burton J, Bye JM, Carder C, Carrel L, Chako J, Chapman JC, Chavez D, Chen E, Chen G, Chen Y, Chen Z, Chinault C, Ciccodicola A, Clark SY, Clarke G, Clee CM, Clegg S, Clerc-Blankenburg K, Clifford K, Cobley V, Cole CG, Conquer JS, Corby N, Connor RE, David R, Davies J, Davis C, Davis J, Delgado O, Deshazo D, Dhami P, Ding Y, Dinh H, Dodsworth S, Draper H, Dugan-Rocha S, Dunham A, Dunn M, Durbin KJ, Dutta I, Eades T, Ellwood M, Emery-Cohen A, Errington H, Evans KL, Faulkner L, Francis F, Frankland J, Fraser AE, Galgoczy P, Gilbert J, Gill R, Glöckner G, Gregory SG, Gribble S, Griffiths C, Grocock R, Gu Y, Gwilliam R, Hamilton C, Hart EA, Hawes A, Heath PD, Heitmann K, Hennig S, Hernandez J, Hinzmann B, Ho S, Hoffs M, Howden PJ, Huckle EJ, Hume J, Hunt PJ, Hunt AR, Isherwood J, Jacob L, Johnson D, Jones S, de Jong PJ, Joseph SS, Keenan S, Kelly S, Kershaw JK, Khan Z, Kioschis P, Klages S, Knights AJ, Kosiura A, Kovar-Smith C, Laird GK, Langford C, Lawlor S, Leversha M, Lewis L, Liu W, Lloyd C, Lloyd DM, Loulseged H, Loveland JE, Lovell JD, Lozado R, Lu J, Lyne R, Ma J, Maheshwari M, Matthews LH, McDowall J, McLaren S, McMurray A, Meidl P, Meitinger T, Milne S, Miner G, Mistry SL, Morgan M, Morris S, Müller I, Mullikin JC, Nguyen N, Nordsiek G, Nyakatura G, O'Dell CN, Okwuonu G, Palmer S, Pandian R, Parker D, Parrish J, Pasternak S, Patel D, Pearce AV, Pearson DM, Pelan SE, Perez L, Porter KM, Ramsey Y, Reichwald K, Rhodes S, Ridler KA, Schlessinger D, Schueler MG, Sehra HK, Shaw-Smith C, Shen H, Sheridan EM, Shownkeen R, Skuce CD, Smith ML, Sotheran EC, Steingruber HE, Steward CA, Storey R, Swann RM, Swarbreck D, Tabor PE, Taudien S, Taylor T, Teague B, Thomas K, Thorpe A, Timms K, Tracey A, Trevanion S, Tromans AC, d'Urso M, Verduzco D, Villasana D, Waldron L, Wall M, Wang Q, Warren J, Warry GL, Wei X, West A, Whitehead SL, Whiteley MN, Wilkinson JE, Willey DL, Williams G, Williams L, Williamson A, Williamson H, Wilming L, Woodmansey RL, Wray PW, Yen J, Zhang J, Zhou J, Zoghbi H, Zorilla S, Buck D, Reinhardt R, Poustka A, Rosenthal A, Lehrach H, Meindl A, Minx PJ, Hillier LW, Willard HF, Wilson RK, Waterston RH, Rice CM, Vaudin M, Coulson A, Nelson DL, Weinstock G, Sulston JE, Durbin R, Hubbard T, Gibbs RA, Beck S, Rogers J, Bentley DR.** The DNA sequence of the human X chromosome. *Nature* 434: 325–337, 2005.
225. **Ross RA, Spengler BA, Biedler JL.** Coordinate morphological and biochemical interconversion of human neuroblastoma cells. *J Natl Cancer Inst* 71: 741–747, 1983.
226. **Rousell J, Haddad EB, Mak JC, Barnes PJ.** Transcriptional down-regulation of m2 muscarinic receptor gene expression in human embryonic lung (HEL 299) cells by protein kinase C. *J Biol Chem* 270: 7213–7218, 1995.
227. **Ryan MJ, Johnson G, Kirk J, Fuerstenberg SM, Zager RA, Torok-Storb B.** HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney. *Kidney Int* 45: 48–57, 1994.
228. **Ryberg D, Hewer A, Phillips DH, Haugen A.** Different susceptibility to smoking-induced DNA damage among male and female lung cancer patients. *Cancer Res* 54: 5801–5803, 1994.
229. **Santoli D, Yang YC, Clark SC, Kreider BL, Caracciolo D, Rovera G.** Synergistic and antagonistic effects of recombinant human interleukin (IL) 3, IL-1 alpha, granulocyte and macrophage colony-stimulating factors (G-CSF and M-CSF) on the growth of GM-CSF-dependent leukemic cell lines. *J Immunol* 139: 3348–3354, 1987.
230. **Sasaki S, Shimokawa H.** The amelogenin gene. *Int J Dev Biol* 39: 127–133, 1995.
231. **Scherer WF, Syverton JT, Gey GO.** Studies on the propagation in vitro of poliomyelitis viruses. IV. Viral multiplication in a stable strain of human malignant epithelial cells (strain HeLa) derived from an epidermoid carcinoma of the cervix. *J Exp Med* 97: 695–710, 1953.
232. **Schmidt M, Deschner EE, Thaler HT, Clements L, Good RA.** Gastrointestinal cancer studies in the human to nude mouse heterotransplant system. *Gastroenterology* 72: 829–837, 1977.
233. **Schneider-Gadicke A, Beer-Romero P, Brown LG, Nussbaum R, Page DC.** ZFX has a gene structure similar to ZFY, the putative human sex determinant, and escapes X inactivation. *Cell* 57: 1247–1258, 1989.
234. **Schneider U, Schwenk HU, Bornkamm G.** Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. *Int J Cancer* 19: 621–626, 1977.
235. **Schoumacher RA, Ram J, Iannuzzi MC, Bradbury NA, Wallace RW, Hon CT, Kelly DR, Schmid SM, Gelder FB, Rado TA, Frizzell RA.** A cystic fibrosis pancreatic adenocarcinoma cell line. *Proc Natl Acad Sci USA* 87: 4012–4016, 1990.
236. **Seifarth JE, McGowan CL, Milne KJ.** Sex and life expectancy. *Gend Med* 9: 390–401, 2012.
237. **Sekido R, Lovell-Badge R.** Sex determination and SRY: down to a wink and a nudge? *Trends Genet* 25: 19–29, 2009.
238. **Sekiguchi M, Sakakibara K, Fujii G.** Establishment of cultured cell lines derived from a human gastric carcinoma. *Jpn J Exp Med* 48: 61–68, 1978.
239. **Shay JW, Zou Y, Hiyama E, Wright WE.** Telomerase and cancer. *Hum Mol Genet* 10: 677–685, 2001.
240. **Shinka T, Naroda T, Tamura T, Sasahara K, Nakahori Y.** A rapid and simple method for sex identification by heteroduplex analysis, using denaturing high-performance liquid chromatography (DHPLC). *J Hum Genet* 46: 263–266, 2001.
241. **Shriver SP, Bourdeau HA, Gubish CT, Tirpak DL, Davis AL, Luketich JD, Siegfried JM.** Sex-specific expression of gastrin-releasing peptide receptor: relationship to smoking history and risk of lung cancer. *J Natl Cancer Inst* 92: 24–33, 2000.
242. **Shulman LM, Bhat V.** Gender disparities in Parkinson's disease. *Expert Rev Neurother* 6: 407–416, 2006.
243. **Siciliano MJ, Barker PE, Cailleau R.** Mutually exclusive genetic signatures of human breast tumor cell lines with a common chromosomal marker. *Cancer Res* 39: 919–922, 1979.
244. **Simunovic F, Yi M, Wang Y, Stephens R, Sonntag KC.** Evidence for gender-specific transcriptional profiles of nigral dopamine neurons in Parkinson disease. *PLos One* 5: e8856, 2010.
245. **Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischauf AM, Lovell-Badge R, Goodfellow PN.** A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* 346: 240–244, 1990.

246. **Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, Repping S, Pyntikova T, Ali J, Bieri T, Chinwalla A, Delehaunty A, Delehaunty K, Du H, Fewell G, Fulton L, Fulton R, Graves T, Hou SF, Latrielle P, Leonard S, Mardis E, Maupin R, McPherson J, Miner T, Nash W, Nguyen C, Ozersky P, Pepin K, Rock S, Rohlfing T, Scott K, Schultz B, Strong C, Tin-Wollam A, Yang SP, Waterston RH, Wilson RK, Rozen S, Page DC.** The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 423: 825–837, 2003.
247. **Skloot R.** *The Immortal Life of Henrietta Lacks*. New York: Broadway Paperbacks, 2011.
248. **Skubitz KM, Pessano S, Bottero L, Ferrero D, Rovera G, August JT.** Human granulocyte surface molecules identified by murine monoclonal antibodies. *J Immunol* 131: 1882–1888, 1983.
249. **Smahi A, Courtois G, Vabres P, Yamaoka S, Heuertz S, Munnich A, Israel A, Heiss NS, Klauck SM, Kioschis P, Wiemann S, Poustka A, Esposito T, Bardaro T, Gianfrancesco F, Ciccodicola A, D'Urso M, Woffendin H, Jakins T, Donnai D, Stewart H, Kenwrick SJ, Aradhya S, Yamagata T, Levy M, Lewis RA, Nelson DL.** Genomic rearrangement in NEMO impairs NF-kappaB activation and is a cause of incontinentia pigmenti. The International Incontinentia Pigmenti (IP) Consortium. *Nature* 405: 466–472, 2000.
250. **Soule HD, Vazguez J, Long A, Albert S, Brennan M.** A human cell line from a pleural effusion derived from a breast carcinoma. *J Natl Cancer Inst* 51: 1409–1416, 1973.
251. **Stern C.** The problem of complete Y-linkage in man. *Am J Hum Genet* 9: 147–166, 1957.
252. **Stich HF, Vanhoosier GL, Trentin JJ.** Viruses and mammalian chromosomes; chromosome aberrations by human adenovirus type 12. *Exp Cell Res* 34: 400–403, 1964.
253. **Stindl R.** Tying it all together: telomeres, sexual size dimorphism and the gender gap in life expectancy. *Med Hypotheses* 62: 151–154, 2004.
254. **Stone KR, Mickey DD, Wunderli H, Mickey GH, Paulson DF.** Isolation of a human prostate carcinoma cell line (DU 145). *Int J Cancer* 21: 274–281, 1978.
255. **Stoner GD, Kaighn ME, Reddel RR, Resau JH, Bowman D, Naito Z, Matsukura N, You M, Galati AJ, Harris CC.** Establishment and characterization of SV40 T-antigen immortalized human esophageal epithelial cells. *Cancer Res* 51: 365–371, 1991.
256. **Sullivan KM, Mannucci A, Kimpton CP, Gill P.** A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. *Biotechniques* 15: 636–638, 640–631, 1993.
257. **Syms AJ, Norris JS, Smith RG.** Proliferation of a highly androgen-sensitive ductus deferens cell line (DDT1MF-2) is regulated by glucocorticoids and modulated by growth on collagen. *In Vitro* 19: 929–936, 1983.
258. **Tashjian AH Jr.** Clonal strains of hormone-producing pituitary cells. *Methods Enzymol* 58: 527–535, 1979.
259. **Tashjian AH Jr, Yasumura Y, Levine L, Sato GH, and Parker ML.** Establishment of clonal strains of rat pituitary tumor cells that secrete growth hormone. *Endocrinology* 82: 342–352, 1968.
260. **Taylor KE, Vallejo-Giraldo C, Schaible NS, Zakeri R, Miller VM.** Reporting of sex as a variable in cardiovascular studies using cultured cells. *Biol Sex Differ* 2: 11, 2011.
261. **Thummel KE.** Gut instincts: CYP3A4 and intestinal drug metabolism. *J Clin Invest* 117: 3173–3176, 2007.
262. **Tom BH, Rutzky LP, Jakstys MM, Oyasu R, Kaye CI, Kahan BD.** Human colonic adenocarcinoma cells. I. Establishment and description of a new line. *In Vitro* 12: 180–191, 1976.
263. **Toulouse A, Sullivan AM.** Progress in Parkinson's disease-where do we stand? *Prog Neurobiol* 85: 376–392, 2008.
264. **Tsuchiya S, Kobayashi Y, Goto Y, Okumura H, Nakae S, Konno T, Tada K.** Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. *Cancer Res* 42: 1530–1536, 1982.
265. **Turner T.** Development of the polio vaccine: a historical perspective of Tuskegee University's role in mass production and distribution of HeLa cells. *J Health Care Poor Underserved* 23: 5–10, 2012.
266. **Vaccarino V, Parsons L, Every NR, Barron HV, Krumholz HM.** Sex-based differences in early mortality after myocardial infarction. National Registry of Myocardial Infarction 2 Participants. *N Engl J Med* 341: 217–225, 1999.
267. **Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, Turnbull A, Singh A, Joubran J, Hazlewood A, Zhou J, McCartney J, Arumugam V, Decker C, Yang J, Young C, Olson ER, Wine JJ, Frizzell RA, Ashlock M, Negulescu P.** Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. *Proc Natl Acad Sci USA* 106: 18825–18830, 2009.
268. **Van Goor F, Hadida S, Grootenhuis PD, Burton B, Stack JH, Straley KS, Decker CJ, Miller M, McCartney J, Olson ER, Wine JJ, Frizzell RA, Ashlock M, Negulescu PA.** Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. *Proc Natl Acad Sci USA* 108: 18843–18848, 2011.
269. **Van Goor F, Straley KS, Cao D, Gonzalez J, Hadida S, Hazlewood A, Joubran J, Knapp T, Makings LR, Miller M, Neuberger T, Olson E, Panchenko V, Rader J, Singh A, Stack JH, Tung R, Grootenhuis PD, Negulescu P.** Rescue of ΔF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. *Am J Physiol Lung Cell Mol Physiol* 290: L1117–L1130, 2006.
270. **Vina J, Borras C, Gambini J, Sastre J, Pallardo FV.** Why females live longer than males: control of longevity by sex hormones. *Sci Aging Knowledge Environ* 2005: pe17, 2005.
271. **von Kleist S, Chany E, Burtin P, King M, Fogh J.** Immunohistology of the antigenic pattern of a continuous cell line from a human colon tumor. *J Natl Cancer Inst* 55: 555–560, 1975.
272. **Wang B, Zhou SF.** Synthetic and natural compounds that interact with human cytochrome P450 1A2 and implications in drug development. *Curr Med Chem* 16: 4066–4218, 2009.
273. **Wang D, Christensen K, Chawla K, Xiao G, Krebsbach PH, Franceschi RT.** Isolation and characterization of MC3T3–E1 preosteoblast subclones with distinct in vitro and in vivo differentiation/mineralization potential. *J Bone Miner Res* 14: 893–903, 1999.
274. **Wang H, Schumacher AE, Levitz CE, Mokdad AH, Murray CJ.** Left behind: widening disparities for males and females in US county life expectancy, 1985–2010. *Popul Health Metr* 11: 8, 2013.
275. **Watanabe M, Zinn AR, Page DC, Nishimoto T.** Functional equivalence of human X- and Y-encoded isoforms of ribosomal protein S4 consistent with a role in Turner syndrome. *Nat Genet* 4: 268–271, 1993.
276. **Weiss A, Wiskocil RL, Stobo JD.** The role of T3 surface molecules in the activation of human T cells: a two-stimulus requirement for IL 2 production reflects events occurring at a pre-translational level. *J Immunol* 133: 123–128, 1984.
277. **Willard HF.** The sex chromosomes and X-chromosome inactivation. In: *The Metabolic Basis of Inherited Disease*, edited by Scriver CR, Beaudet AL, Sly D, Valle D, Childs B, and Vogelstein B. New York: McGraw-Hill, 2000.
278. **Wizemenn TM, Pardue ML.** *Exploring the Biological Contributions to Human Health: Does Sex Matter?* Washington, DC: Natl Acad Press, 2001.
279. **Woolford JL Jr.** The structure and biogenesis of yeast ribosomes. *Adv Genet* 29: 63–118, 1991.
280. **Wooten GF, Currie LJ, Bovbjerg VE, Lee JK, Patrie J.** Are men at greater risk for Parkinson's disease than women? *J Neurol Neurosurg Psychiatry* 75: 637–639, 2004.
281. **Wright V, Peng H, Usas A, Young B, Gearhart B, Cummins J, Huard J.** BMP4-expressing muscle-derived stem cells differentiate into osteogenic lineage and improve bone healing in immunocompetent mice. *Mol Ther* 6: 169–178, 2002.
282. **Wu JC, Merlino G, Fausto N.** Establishment and characterization of differentiated, nontransformed hepatocyte cell lines derived from mice transgenic for transforming growth factor alpha. *Proc Natl Acad Sci USA* 91: 674–678, 1994.
283. **Wutz A.** Gene silencing in X-chromosome inactivation: advances in understanding facultative heterochromatin formation. *Nat Rev Genet* 12: 542–553, 2011.
284. **Xu KP, Yadav BR, King WA, Betteridge KJ.** Sex-related differences in developmental rates of bovine embryos produced and cultured in vitro. *Mol Reprod Dev* 31: 249–252, 1992.
285. **Yaffe D, Saxel O.** A myogenic cell line with altered serum requirements for differentiation. *Differentiation* 7: 159–166, 1977.
286. **Yaffe D, Saxel O.** Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. *Nature* 270: 725–727, 1977.
287. **Yamane H, Kiura K, Tabata M, Bessho A, Tsuchida T, Motoda K, Hiraki A, Ueoka H, Harada M.** Small cell lung cancer can express CD34 antigen. *Anticancer Res* 17: 3627–3632, 1997.
288. **Yamazaki T, Yokoyama T, Akatsu H, Tukiyama T, Tokiwa T.** Phenotypic characterization of a human synovial sarcoma cell line, SW982, and its response to dexamethasone. *In Vitro Cell Dev Biol Anim* 39: 337–339, 2003.

289. **Yang F, Babak T, Shendure J, Disteche CM.** Global survey of escape from X inactivation by RNA-sequencing in mouse. *Genome Res* 20: 614–622, 2010.
290. **Yang X, Schadt EE, Wang S, Wang H, Arnold AP, Ingram-Drake L, Drake TA, Lusis AJ.** Tissue-specific expression and regulation of sexually dimorphic genes in mice. *Genome Res* 16: 995–1004, 2006.
291. **Yee C, Krishnan-Hewlett I, Baker CC, Schlegel R, Howley PM.** Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. *Am J Pathol* 119: 361–366, 1985.
292. **Yufu Y, Goto T, Choi I, Uike N, Kozuru M, Ohshima K, Taniguchi T, Motokura T, Yatabe Y, Nakamura S.** A new multiple myeloma cell line, MEF-1, possesses cyclin D1 overexpression and the p53 mutation. *Cancer* 85: 1750–1757, 1999.
293. **Yunis AA, Arimura GK, Russin DJ.** Human pancreatic carcinoma (MIA PaCa-2) in continuous culture: sensitivity to asparaginase. *Int J Cancer* 19: 128–135, 1977.
294. **Zabner J, Karp P, Seiler M, Phillips SL, Mitchell CJ, Saavedra M, Welsh M, Klingelhutz AJ.** Development of cystic fibrosis and noncystic fibrosis airway cell lines. *Am J Physiol Lung Cell Mol Physiol* 284: L844–L854, 2003.
295. **Zang EA, Wynder EL.** Differences in lung cancer risk between men and women: examination of the evidence. *J Natl Cancer Inst* 88: 183–192, 1996.
296. **Zanger UM, Schwab M.** Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* 138: 103–141, 2013.
297. **Zeitlin PL, Lu L, Rhim J, Cutting G, Stetten G, Kieffer KA, Craig R, Guggino WB.** A cystic fibrosis bronchial epithelial cell line: immortalization by adeno-12-SV40 infection. *Am J Respir Cell Mol Biol* 4: 313–319, 1991.
298. **Zhang W, Bleibel WK, Roe CA, Cox NJ, Eileen Dolan M.** Gender-specific differences in expression in human lymphoblastoid cell lines. *Pharmacogenet Genomics* 17: 447–450, 2007.
299. **Zorgdrager A, De Keyser J.** The premenstrual period and exacerbations in multiple sclerosis. *Eur Neurol* 48: 204–206, 2002.