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VIEWPOINT

Insufficient sex description of cells supplied by commercial vendors

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EVIDENCE SUPPORTING sex as a biologically important variable in human and animal studies has been accumulating over recent decades (1, 4, 6–8, 10). As a result, the necessity of including female as well as male subjects in clinical studies has been widely recognized (1, 10). In animal experiments, the inclusion of both sexes is encouraged to monitor sex-based differences (7). In addition, funding agencies such as the National Institutes of Health (NIH) require investigators to include both male and female participants when designing their research (see http://grants.nih.gov/grants/funding/women_min/guidelines_amended_10_2001.htm). Accordingly, there are now more preclinical studies and clinical trials that include participants of both sexes and report outcomes stratified by sex (3, 13), even though further efforts are required to engrave sex as a key factor in experimental design (9).

Basic science researchers often use cell lines and primary cells in their proof-of-concept experiments and investigations of biological mechanisms. Results from such studies are often used as the basis for developing new drugs, therapeutic modalities, and diagnostic methods for translation to human medicine. However, the sex of in vitro cell lines, primary cells, and stem cells is often ignored.

Most scientific articles do not properly report the sex of cells (5, 12, 14). Academic journals, with the exception of a few, do not have guidelines for reporting the sex of cells used in research, in contrast to the fact that many journals ask researchers to report the sex of human participants and experimental animals. Taylor et al. (14) reviewed articles published in 2010 to determine the extent to which the sex of cells was reported in cardiovascular studies and found that, among 191 articles published in top cardiovascular journals, only 45 articles (23.6%) reported cell sex. Among these studies, most (68.9%) used only male cells, and none exclusively used female cells (14). This situation is not limited to any one research field. Shah et al. (12) reported that only 25% of 100 articles randomly selected from *American Journal of Physiology-Cell Physiology* published in 2013 described the sex of cells used in experiments.

As many researchers use commercially available cells without further characterization, they often do not know the sex of the cells unless the vendors provide the information. To investigate how major cell banks that supply cells worldwide describe the sex of their cells, we analyzed in December 2013 the homepages of three representative cell banks: American Type Culture Collection (ATCC; http://www.atcc.org), European Collection of Cell Cultures (ECACC; http://www.phe-culturecollections.org.uk/collections/ecacc.aspx), and Japanese Collection of Research Bioresources (JCRB; https://cellbank.nibio.go.jp/english/).

Figure 1 shows that the male-to-female ratio of human cell lines of the ATCC was nearly even, but >20% of cell lines lacked sex description. Cell lines of undefined sex were much more common in non-human animal cell lines, with 92% of mouse and 83% of rat cell lines being sold without sex identification (Fig. 1). The male-to-female ratio of cells was also roughly even for mouse cell lines, but male rat cell lines were twice as prevalent as female rat cell lines. Female other animal cell lines provided by ATCC outnumbered male cell lines by a factor of two (Fig. 1). This can be attributed to the fact that most hamster cell lines (38 out of 42) were originated from the ovary. For the ECACC, the sex of human cell lines was relatively well known, and the male-to-female ratio of cell lines was almost even; however, descriptions of sex were virtually nonexistent for animal cell lines (Fig. 1). For the JCRB, ~40% of human cell lines lacked sex description and >77% of animal cell lines lacked sex description (Fig. 1).

The ATCC provided sex information for all human primary cells (Table 1), and male cells were more than twice as prevalent as female cells. Sexes of some human primary cells from ATCC were flexible depending on the batches. However, sex description was missing for all of the human and animal primary cells provided by the ECACC and the JCRB cell banks (Table 1).

Regarding human stem cells, the ATCC provided 14 male cells, 2 undefined cells, and 3 flexible cells depending on the batches, but no female cells were available (Table 2). Regarding mouse stem cells, the ATCC provided 11 undefined cells, 9 male cells, and 8 cells consisting of mixed male and female cells, but no female cells were provided. The ECACC provided 15 human and 33 animal stem cells, and ~75% lacked sex identification (Table 2). Human stem cells provided by the JCRB cell bank consisted of 1 male cell, 18 female cells, and 22 undefined cells (Table 2). All mouse stem cells provided by the JCRB were of undefined sex, whereas sex was specified for stem cells from other species (Table 2).

The ATCC provides tumor cell panels, which are groups of cell lines consisting of three to nine species that best represent tumor categories and their special characteristics, including any mutations. After excluding the breast and gynecologic cancer cell panels, which consist of all female cells, the remaining 24 tumor cell panels were categorized into 13 groups according to their tissue type to analyze their sex composition (Fig. 2). We found that the colon, liver, lung, and

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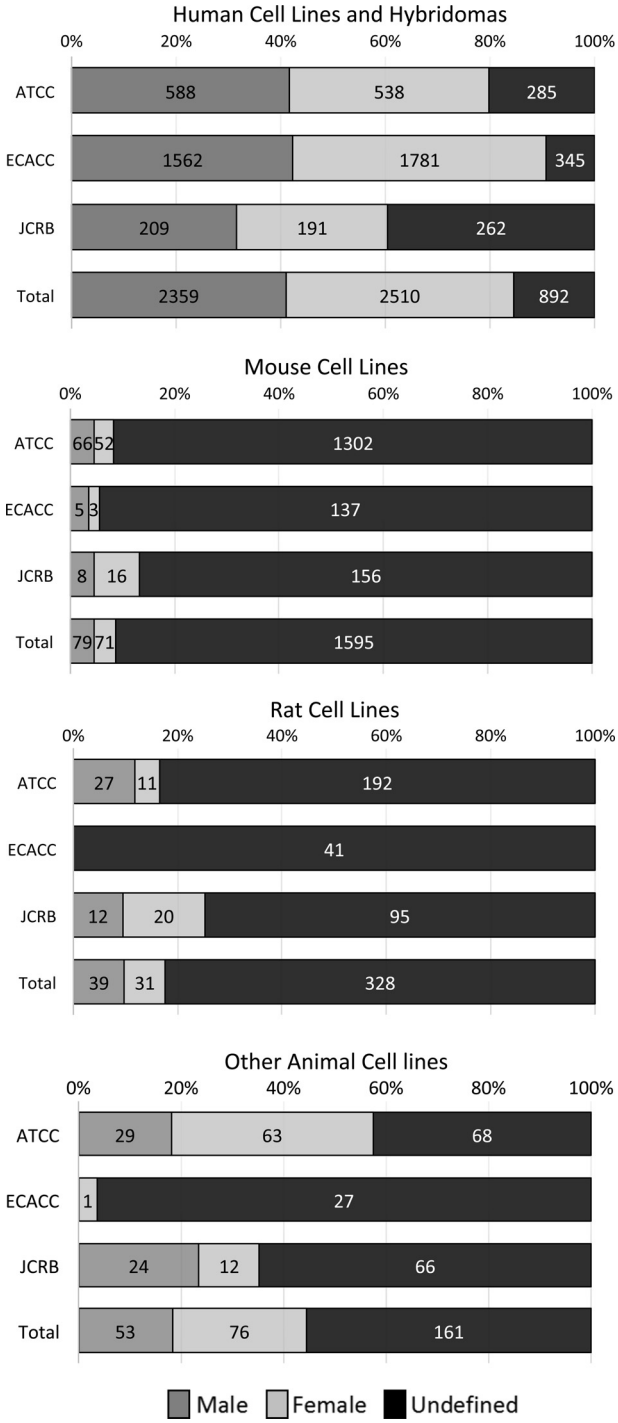

Fig. 1. Sex of cell lines and hybridomas from commercial vendors. Cell lines from the American Type Culture Collection (ATCC), European Collection of Cell Cultures (ECACC), and Japanese Collection of Research Bioresources (JCRB) were categorized according to their species of origin (human, mouse, rat, or other animal) and then analyzed for their sex distribution. Other animal cell lines were from hamster, cat, monkey, rabbit, pig, mink, zebrafish, horse, and chicken (ATCC); hamster, cat, sheep, monkey, dog, insect, cow, fish, and rabbit (ECACC); and hamster, monkey, cow, cat, chicken, chimpanzee, dog, cotton-top tamarin, goat, guinea pig, Indian muntjak, marsupial, mink, mosquito, mouse-human hybridoma, mouse-rat hybridoma, pig, pygmy mouse, and rabbit (JCRB). Number of cells belonging to each category is shown in the bar.

Table 1. *Sex of primary cells*

Source	Species	Male	Female	Undefined	Lot (batch)-Specific	Total
ATCC	Human	22[a]	9[b]	0	20	51
ECACC	Human	0	0	57	0	57
	Animal[c]	0	0	44	0	44
JCRB	Human	0	0	1,387[d]	0	1,387[d]
	Animal[e]	0	0	5	0	5

[a]Eight cells were from foreskin. [b]Two cells were from mammary glands. [c]Cells were from mouse (1), rat (14), pig (6), dog (7), cat (4), cow (10), rabbit (1), and chicken (1). [d]1,375 cells were skin fibroblasts. [e]Cells were from mouse (3) and cow (2).

lymphoma tumor cell panels contained cell lines of unknown sex. In addition, the majority of tumor cell panels had more male cell lines than female cell lines. Furthermore, two brain tumor cell panels contained only male cells and no female cells. Overall, tumor cell panels from the ATCC consisted of 65% male cells, 32% female cells, and 3% undefined cells (Fig. 2).

We found that ~15.5% of human cell lines were sold without sex identification. Animal cell lines lacked sex identification more often than human cell lines. Over 91% of mouse cell lines and over 82% of rat cell lines provided by the commercial vendors we analyzed had no defined sex. Although the male-to-female ratios of cells were rather even for both human and animal cell lines, this ratio was not balanced for primary cells and stem cells, for which male cells were generally provided more frequently than female cells.

The perception of sex as an important biological factor and its integration into research design and analysis can directly improve our understanding of the etiology of diseases, which could increase the effectiveness of treatments and minimize side effects (for example, see http://genderedinnovations.stanford.edu/). By indicating the sex of cells they provide, commercial vendors could help researchers evaluate sex as a possible variable in basic biomedical research. In particular, it would be helpful for cell vendors to provide sex-based search engines that can be easily used by researchers. This may help researchers design experiments using a well-balanced sex distribution of cells, resulting in more accurate and unbiased outcomes.

Recently, the NIH announced that, effective October 2014, grant applicants must balance the sex of animals and cells they intend to study (2), which encourages the consideration of cell sex in preclinical study design and analysis. In parallel, NIH also plans to collaborate with journal publishers to require

Table 2. *Sex of stem cells*

Source	Species	Male	Female	Undefined	Others	Total
ATCC	Human	14[a]	0	2	3[b]	19
	Mouse	9	0	11	8[c]	28
ECACC	Human	3	1	11		15
	Animal[d]	4	4	25		33
JCRB	Human	1	18	22		41
	Mouse	0	0	111[e]		111
	Other	6	1	0		7

[a]Six cells were from foreskin. [b]Cells were lot or batch specific. [c]Cells were a mix of male and female cells. [d]Cells were from mouse (25), rat (3), cow (1), fish (1), rabbit (1), dog (1), and cat (1). [e]Cells were mainly embryogenic stem cells.

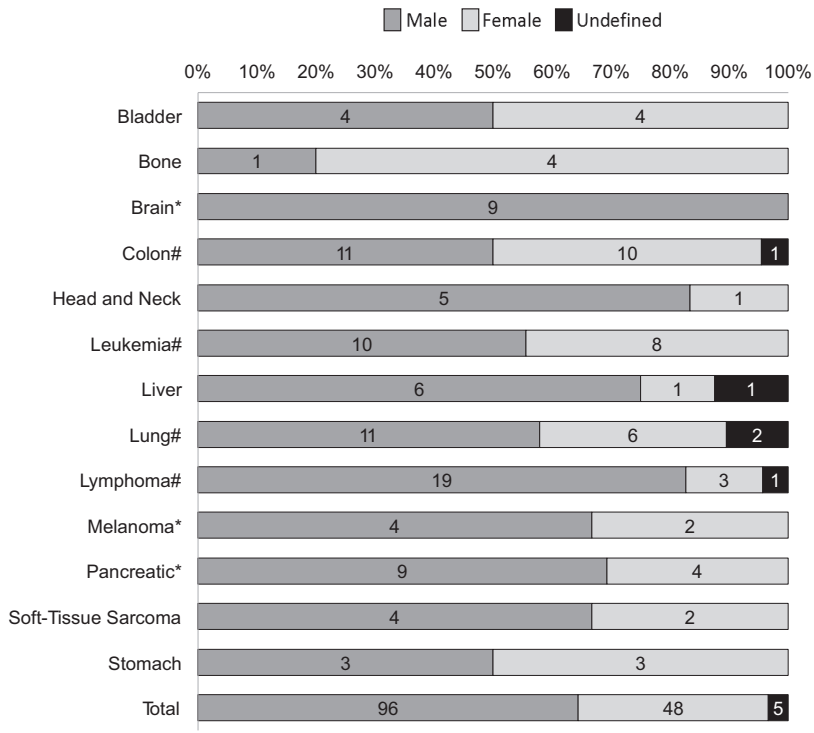

Fig. 2. Sex of cell lines included in ATCC tumor cell panels. To simplify the results, two (*) or three (#) different tumor cell panels were combined if they were from the same tissue origin. For example, the brain cancer cell panel was combined with the brain (glioma) tumor cell panel and shown as "Brain," and colon cancer panels 1 and 2 were combined with colon cancer p53 hotspot mutation cell panel and shown as "Colon." Breast and gynecologic cancer cell panels were excluded because they include only female cells. Number of cells belonging to each category is shown in the bar.

researchers to clearly state the sex of animals and cells used in their experiments. Thus, knowing the sex of the cells is becoming essential. In cases the sex of a cell line already in use is unknown, the polymerase chain reaction (PCR) method can be utilized to rapidly identify cell sex. This method takes advantage of the fact that the amelogenin gene (*AMELX* and *AMELY*) exhibits different lengths in X and Y chromosomes (11) and can be performed either by the investigators themselves or a third-party company to identify the sex of cell lines.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

H.Y.P. and S.K.L. conception and design of research; J.H.P. and S.K.L. prepared figures; M.N.P. and S.K.L. drafted manuscript; M.N.P. and S.K.L. edited and revised manuscript; M.N.P., J.H.P., H.Y.P., and S.K.L. approved final version of manuscript.

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