Brief/Technical Note

The Gender of Cell Lines Matters When Screening for Novel Anti-Cancer Drugs

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Received 20 February 2014; accepted 12 May 2014; published online 30 May 2014

Abstract. Current reports indicated that the gender origin of cells is important in all facets of experimental biology. To explore this matter using an anticancer high throughput screening platform, seven male- and seven female-derived human cell lines, six from cancer patients in each group, were exposed to 81 novel cytotoxins. In this screen, the findings revealed that 79 out of 81 of the compounds consistently inflicted higher levels of toxicity towards male derived cells, emphasizing that there is indeed a gender-related difference in cell sensitivity to these anti-neoplastic agents. This gender-related drug sensitivity and toxicity explored at the molecular and cellular level emerged from a drug discovery enterprise.

KEY WORDS: cancer; cytotoxicity; drug discovery; high throughput screening.

INTRODUCTION

Recent articles captured our attention regarding the relevance of gender origin of biological specimens used in all facets of experimental biology (1,2). Together, these reports embraced numerous publications indicating that cells behave differently depending on their male or female origin, under a variety of stimuli/conditions. A few examples are as follows: After 14 days of incubation, the total number of surviving primary cortical neurons was significantly higher in female rat cultures, as compared with male rat cultures (3). The resistance of female rats to gut injury and gut-induced distant organ injury was greater as compared with male rats (4). Moreover, using whole mouse embryos before sexual differentiation, Penaloza *et al.* (5) found differential gene expression between female and male derived cells, in response to ethanol, camptothecin, or influenza A virus, indicating that the gender dimorphic response is a general phenomenon and apparently independent of hormonal contribution (5). In this context, it is crucial to elucidate whether gender-related differences can affect the results in cytotoxicity assays commonly used in drug discovery to detect promising anti-cancer drugs. To examine this possibility, 81 cytotoxins were evaluated against seven male- and seven female-derived human cell lines.

MATERIALS AND METHODS

Cells were cultured following the American Tissue Culture Collection guidelines (ATCC; Manassas, VA; Table I). The ATCC cell repository maintains an extensive database for each individual cell line, which can be accessed online (<http://www.atcc.org>). Adherent and non-adherent cells were seeded at a density of 10,000 and 15,000 cells per well, respectively, in 200 μ l of growth media using 96-well plate format. Cells were exposed to 1 μ M of each compound and the toxicity recorded after 24 h. Cytotoxicity was determined *via* the MTS-based assay (Promega) or the live-cell differential nuclear staining imaging-based assay, which has been validated for high throughput screening (HTS) initiatives (6). The procedures previously described (6) were followed to guarantee high cell viability. Each experimental measurement was performed in triplicate, and its average was calculated. Since the experimental compounds were dissolved in dimethyl sulfoxide, cells treated with this solvent (0.5% *v/v*) were included in each plate and the cell death average value was subtracted from each compound-treated value to improve accuracy and remove cell death background. Plates exhibiting percentages of less than 95% viability for untreated controls were repeated. Additionally, the averages of cytotoxicity obtained from each compound to each cell line were added according with the gender group and a new average was calculated for comparison purposes. The cell death percentage between the two gender groups for each compound was compared side by side.

RESULTS

The cytotoxins used in this study are conjugated unsaturated ketones and 78/81 compounds have the

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Table I. Cell Lines^a Clustered by Gender Origin, Male and Female, Utilized in the Screen of 81 Cytotoxins

Cell Line	Cancer Type	Age of Donor
Male origin		
RAMOS	Lymphoma	3 ^b
COLO 205	Colon	70
Raji	Lymphoma	11
LAPC-4	Prostate	— ^c
Jurkat	Leukemia	14
Nalm-6	Leukemia	19
Hs-27	Non-cancerous from foreskin	Newborn
Female Origin		
HT-29	Colon	44 ^b
HCC70	Breast	49
CEM	Leukemia	4
MDA-231	Breast	51
MDA-468	Breast	51
MCF-7	Breast	69
MCF-10-A	Non-cancerous from breast	36

RAMOS human Caucasian Burkitt's lymphoma, *Colo 205* human Caucasian colon adenocarcinoma, *Raji* human Black, Burkitt's lymphoma, *LAPC-4* Los Angeles prostate cancer, *Jurkat* immortalized line of human T lymphocyte, *Nalm-6* B cell precursor leukemia, *HS-27* Human skin fibroblast cells, *HT-29* Human colon adenocarcinoma grade II cell line, *HCC-70* Human breast cancer cell line, *CEM* lymphoblastic leukemia cells, *MDA-231* human breast cancer cell line, *MCF-7* Michigan Cancer Foundation-7, *MCF-10-A* human breast epithelial cell line

^aIn-depth information on each specific cell line is available online from ATCC webpage (<http://www.atcc.org/>). Six of the seven cell lines for each gender were isolated from human cancer patients whereas Hs27 (human foreskin fibroblast) and MCF-10A (breast epithelial) are human non-cancerous cell lines used as controls

^bYears old

^cThe age of the male patient from which the LAPC-4 cell line was derived was not provided in the original publication (7). This line was isolated using a mouse SCID xenograft mouse model

general structure presented in Fig. 1. The exceptions are the bis-Mannich bases NC901 and NC1114 which are capable of releasing conjugated dienones [$\text{CH}_2=\text{C}(\text{R})-\text{CO}-\text{C}(\text{R})=\text{CH}_2$] and NC1949 which contains the 4-oxo-2-butenyl substituent [$-\text{C}(\text{O})-\text{CH}=\text{CH}-\text{C}(\text{O})-$]. The male

X = N-R, CH₂

R¹ = alkyl, halo, alkoxy, O-aryl, nitro groups

R = H, CH₃, aryl, acyl, phosphate groups

Fig. 1. The general structure of the α,β -unsaturated ketones employed in this study

and female human cell lines used in this study are indicated in Table I, respectively.

Cells derived from males were consistently more sensitive to the majority of experimental compounds (97.5%; 79 out of 81 compounds; see blue bars in Fig. 2b–e). In contrast, only two compounds exhibited slightly more toxicity to cells of female origin (2.5%; Fig. 2a). Based in fold change values, the preferential exerted greater toxicity to male cells, rather than female cells, revealed that six compounds had figures of >3 (7.4%; Fig. 2b), while 17 compounds had a range from 3 to >2 (21.0%; Fig. 2c), and 56 compounds between 2 to >1 (69.1%; Fig. 2d). Comparative analysis of the cytotoxic effects of the experimental compounds on individual non-cancerous control cells, indicated that the percentage of toxicity detected in male Hs27 and female MCF-10A cells was 17.3 and 11.5% ($p < 0.001$), respectively.

To gain an insight on whether the donor's age correlates with cell drug sensitivity, the cytotoxic average of the 81 compounds per cell line was obtained for comparative analysis. Two breast cancer cell lines derived from 51-year-old female donors were compared for their sensitivity to the test compounds and these exhibited 13.97 and 40.05% cytotoxicity on MDA-231 and MDA-468, respectively. This result suggests an interindividual variability in cellular sensitivity to the experimental compounds, implying that this cytotoxic effect is age and tissue independent. However, the correlation between donor's age and cell sensitivity is quite complex and continues undecipherable, requiring additional experimental scrutiny.

DISCUSSION

The compounds used in this study are conjugated ketones except NC 901 and NC 1114 *vide supra*. They are designed to interact with cellular thiols and not amino or hydroxyl groups which are present in nucleic acids. The compounds may therefore be devoid of the genotoxic side effects of a number of anticancer drugs (8). The compounds represented by the general structure in Fig. 1 contain the 1,5-diaryl-3-oxo-1,4-pentadienyl group, and the cytotoxic and anticancer properties of compounds containing this pharmacophore have been reviewed (9).

Our contribution corroborates similar findings and reveals that there is a gender-linked difference in anti-neoplastic cytotoxicity. The results demonstrated that there is a greater sensitivity of the male-derived cells as compared with cells of female origin. This preferential increased sensitivity highlights the necessity to discern the gender source of cells used in HTS initiatives.

The authors hope that this report encourages including the gender origin of the cell lines in publications dealing with cytotoxic bioassays. It is anticipated that a greater understanding of the differences in gender sensitivity to cytotoxins should increase dosing accuracy. Future research is needed to reveal whether this *in vitro* observation occurs in animal models.

Fig. 2. Male origin cells proved to be more sensitive than female derived cells to 79 cytotoxins *in vitro*. Cytotoxicity was grouped by gender-based origin of the cells for each compound and used for comparison purposes. **a** Results obtained with two compounds that were slightly more toxic for female origin cells, rather than male source cells. Averages of cytotoxicity (y-axis) per single compound and same-sex group were assembled as range values of fold change, comparing male- vs. female-derived cells, are depicted as follows: **b** >3.0; **c** between 3.0 and >2.0; and **d** between 2 to >1. Blue bars correspond to male cells, whereas pink bars to female cells

ACKNOWLEDGMENTS

Funding for this work was provided by the NIGMS SCORE grant 1SC3GM103713-01 to R.J.A., as well as a CIHR-RPP Saskatchewan grant to J.R.D and U.D. We thank the Cytometry, Screening and Imaging Core Facility at the University of Texas at El Paso (UTEP) which was supported by a RCMI program grant 8G12MD007592 to the BBRC-UTEP from the NIMHD-NIH. E.R-E, Y.S-V and N.M.O. were supported by NIGMS RISE training grant R25 GM069621-10. N.M.O. was also supported by MARC U*STAR program grant 2T34GM008048.

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