Exploring Applications of Human Primary Cells for Drug Screening in Various Cell Culture Systems

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Abstract #1822

Abstract

Human primary cells are useful pre-clinical models as they more closely mimic the physiology of cells *in vivo* than continuous cell lines. Here, we explored some of the applications of human primary cells for cytotoxicity assays and drug screening. Cancer is a leading cause of female mortality worldwide and gynecologic cancers have a low survival rate. Further, cytotoxicity is a common side effect of all anti-cancer drugs. We investigated the cytotoxic effects of three anti-cancer drugs (alone or in combination) on three types of normal reproductive cells *in vitro*. Primary human uterine fibroblasts, cervical epithelial cells, and vaginal epithelial cells along with cervical (SiHa) and vaginal (VK2/E6E7) epithelial cancer cell lines were treated for two days with topotecan, paclitaxel, cisplatin, or a combination of topotecan and cisplatin at concentrations of 0 µM, 0.1 µM, 1 µM, 10 µM, or 100 µM. Cytotoxicity of these chemotherapeutic drugs was assessed by using Reliablue™ Cell Viability Reagent. Both topotecan and paclitaxel significantly induced cytotoxicity in three types of reproductive primary cells at 0.1 µM, while cisplatin significantly decreased the viability of all cells at 1 µM or 10 µM. Topotecan, when used in combination with cisplatin, resulted to a significant increase in cytotoxicity at 0.1 µM, 1 µM, 10 µM, or 100 µM. Furthermore, SiHa and VK2/E6E7 cancer cell lines were less sensitive to the aforementioned chemotherapeutic drugs compared to primary cells. Three-dimensional (3D) cell culture systems utilizing primary cells may provide more physiologically relevant information and more predictive data in *in vitro* assays. Primary human skeletal muscle cells can contract under physiological conditions and their contractility may change during tissue injury and repair. To validate a 3D muscle contraction assay for drug screening applications, we embedded primary human skeletal muscle cells with a collagen matrix and treated them for 10 days with 10 mM 2,3-butanedione 2-monoxime (BDM), a known inhibitor of skeletal muscle contraction. The spontaneous contraction of untreated human skeletal muscle cells was observed in a time-dependent manner during the BDM treatment. The results from these assay systems demonstrate that various types of human primary cells can be used for pre-clinical applications including drug screening, toxicology, and modeling physiological responses.

Results

Primary Human Skeletal Muscle Cells As an *In Vitro* Model System for Studying Muscle Differentiation and Contraction

Method of Skeletal Muscle Contraction Assay in 3D Culture: (1) Primary Skeletal Muscle Cells were harvested and re-suspended in complete growth medium (Mesenchymal Stem Cell Basal Medium [ATCC® PCS-500-030™] plus Primary Skeletal Muscle Growth Kit [ATCC® PCS-950-040™]). (2) The cell suspension was mixed with cold collagen gel solution. (3) The cell-collagen mixture was seeded into a 24-well plate and incubated at 37ºC for 1 hr. (4) SkMc complete growth media with or without 10 mM 2,3-butanedione monoxime (BDM) was added after collagen gel polymerization. (5) To induce contraction, collagen gels were released from the sides of the wells with a sterile 10 µL pipette tip. (6) Medium was changed on days 3 and 6.

1A

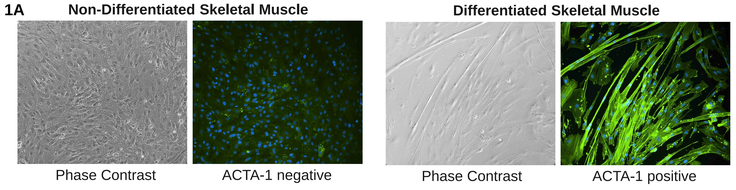
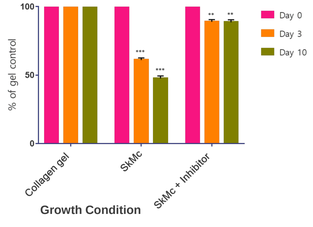


Figure 1A. Immunocytochemistry of non-differentiated and differentiated Primary Skeletal Muscle Cells (SkMC; ATCC® PCS-950-010™) with ACTA-1 antibodies (actin proteins are important for cell movement and muscle fiber contraction). Original magnification, 20x.

1B



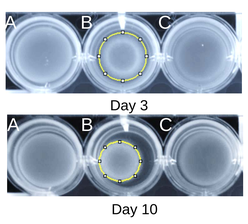


Figure 1B. Skeletal muscle (SkMc) contraction assay. SkMc were embedded with a collagen matrix and treated with or without 10 mM BDM inhibitor for up to 10 days. Significant (***p < 0.001) contraction was observed in SkMc in complete culture medium. SkMc with inhibitor showed less significant (**p < 0.01) contraction. (A) Collagen gel only. (B) SkMc only. (C) SkMc + BDM Inhibitor (**p < 0.01, ***p < 0.001 vs. gel control, Student's T-test).

Drug Screening of Human Primary Cells and Cancer Cell Lines

Method of Toxicity Assay: (1) Primary and continuous human cervical (ATCC® PCS-480-011™ and ATCC® CRL-2616™), primary and continuous vaginal (ATCC® PCS-480-010™ and ATCC® CRL-2616™), and primary uterine (ATCC® PCS-460-010™) cells were seeded in 96-well plates at a density of 30,000 to 40,000 cells/cm². (2) Cells were cultured in their respective expansion media overnight prior to treating with three chemotherapeutic drugs (topotecan, paclitaxel, cisplatin) at 0.1 µM, 1 µM, 10 µM, or 100 µM for two days. (3) Cytotoxicity was assessed by using Reliablue™ Cell Viability Reagent (ATCC® 30-1014™).

2A

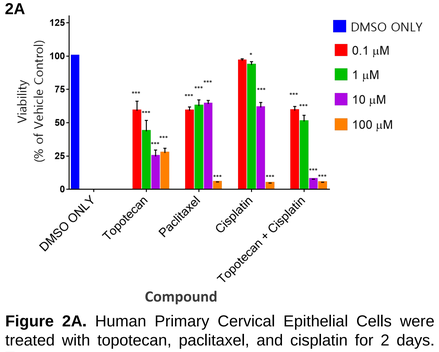


Figure 2A. Human Primary Cervical Epithelial Cells were treated with topotecan, paclitaxel, and cisplatin for 2 days. Topotecan, paclitaxel, and topotecan combined with cisplatin at 0.1 µM, 1 µM, 10 µM, or 100 µM significantly induced cytotoxicity while cisplatin alone significantly induced cytotoxicity at 1 µM, 10 µM, and 100 µM (n=3,*p < 0.05, **p < 0.01, ***p < 0.001 vs. DMSO control, Student's T-test).

2B

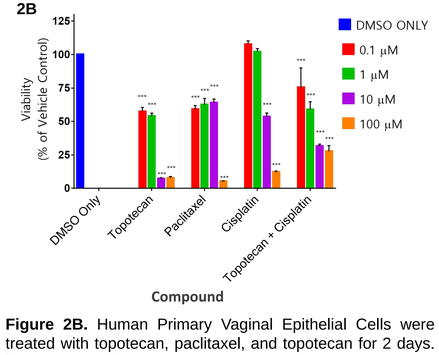


Figure 2B. Human Primary Vaginal Epithelial Cells were treated with topotecan, paclitaxel, and topotecan for 2 days. Topotecan, paclitaxel, and topotecan combined with cisplatin at 0.1 µM, 1 µM, 10 µM, or 100 µM significantly induced cytotoxicity. Cisplatin alone significantly induced cytotoxicity at 10 µM and 100 µM (n=3, ***p < 0.001 vs. DMSO control, Student's T-test).

2C

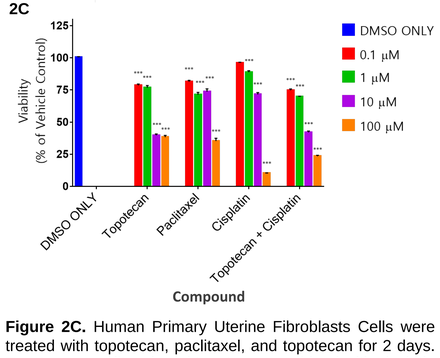


Figure 2C. Human Primary Uterine Fibroblasts Cells were treated with topotecan, paclitaxel, and topotecan for 2 days. Topotecan, paclitaxel, and topotecan combined with cisplatin at 0.1 µM, 1 µM, 10 µM, or 100 µM significantly induced cytotoxicity. Cisplatin alone significantly induced cytotoxicity at 1 µM, 10 µM, and 100 µM (n=3, ***p < 0.001 vs. DMSO control, Student's T-test).

2D

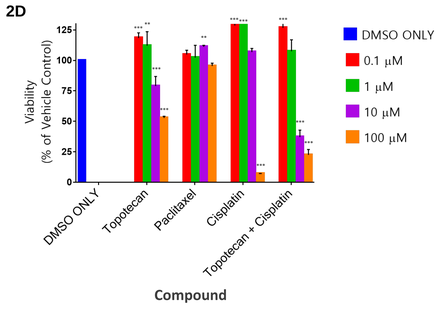


Figure 2D. SiHa cervical cancer epithelial cells were treated with topotecan, paclitaxel, and cisplatin for 2 days. Topotecan at 10 µM or 100 µM significantly induced cytotoxicity while paclitaxel didn't decrease cell viability. Cisplatin significantly induced cytotoxicity only at 100 µM. Topotecan combined with cisplatin at 10 µM or 100 µM significantly induced cytotoxicity (n=3, **p < 0.01, ***p < 0.001 vs. DMSO control, Student's T-test).

2E

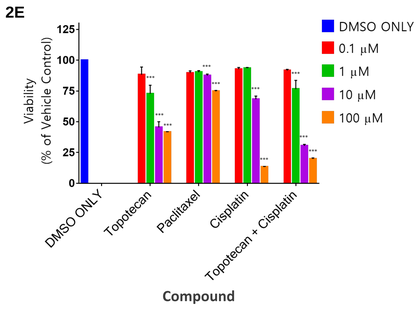


Figure 2E. VK2/E6E7 vaginal mucosal epithelial cancer cells were treated with topotecan, paclitaxel, and cisplatin for 2 days. Topotecan and a combination of topotecan and cisplatin at 1 µM, 10 µM, or 100 µM, significantly induced cytotoxicity. Paclitaxel or cisplatin significantly induced cytotoxicity at 10 µM (n=3, ***p < 0.001 vs. DMSO control, Student's T-test).

Summary

- Human primary cells can be used for pre-clinical applications including drug screening, toxicology, and modeling physiological responses.
- Topotecan, paclitaxel, and cisplatin were toxic to primary cervical and vaginal epithelial cells and primary uterine fibroblast cells.
- *In vitro* muscle contractile function assays are important to characterize SkMc and to demonstrate the efficacy of a muscle treatment such as an inhibitor.

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