

EXPLORING TERATOGENICITY BIOMARKERS IN DENTAL STROMAL CELLS FOR ADVANCING NAM-BASED TOXICOLOGICAL TOOLS

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INTRODUCTION: Exposure to teratogenic agents during pregnancy can harm embryonic development. The OECD 414 (2018) guideline remains the gold standard for DART testing; however, it is resource-intensive, requires nearly 1,000 animals per study, and may not accurately predict human outcomes. These limitations highlight the need for New Approach Methodologies (NAMs) that are human-relevant and animal-free. Identifying reliable teratogenicity biomarkers is essential for developing human-based in vitro models. Dental stromal cells, easily obtained from discarded teeth, offer a promising source of human multipotent stem cells with well-defined biomarker expression.

OBJECTIVE: To develop a teratogen screening platform using dental stromal cells as a human-based model. **MATERIALS AND METHODS:** Stromal cells from the apical papilla (SCAPs) were isolated, cultured, and characterized by flow cytometry. Cells were exposed to classical teratogenic - 5-fluorouracil (0.1–360 µg/mL), methotrexate, and cyclophosphamide (0.1–1000 µg/mL) and the non-teratogenic compound folic acid (0.16–250 µg/mL). CV₈₀ values were determined using the MTT assay. Biomarker expression (Nestin, OCT-3/4, and Stro-1) was assessed at 48 hours, while CD90 and CD105 were evaluated from 24 to 120 hours by flow cytometry. **RESULTS AND CONCLUSIONS:** SCAPs exhibited a positive phenotype for CD90, CD105, Stro-1, Nanog, OCT-³/₄ and Nestin, while CD34 expression was negative. No cytotoxicity was observed at any concentration after 24 hours of exposure to 5-FU, MTX, CTX, or folic acid. However, the CV₈₀ value was determined to be 13.47 µg/mL after 48 hours of exposure to 5-FU. The CV_{80-48h} values for folic acid, MTX, and CTX could not be established. No modulation was observed in the expression of Nestin, OCT-³/₄, Stro-1, or CD90 following exposure to 5-FU. However, a concentration- and time-dependent reduction in CD105 expression was evident when SCAPs were exposed to 5-FU, MTX, and CTX at 72 and 120 hours. In contrast, folic acid did not alter CD105 expression. These findings suggest the potential of human dental stromal cells as a relevant tool for developing NAMs. Additionally, CD105 downregulation may disrupt TGF-β signaling via ALK-1/SMAD 1/5/8, impairing proliferation, migration, and angiogenesis, mechanisms consistent with known teratogenic effects, such as those caused by thalidomide. Therefore, CD105 emerges as a key biomarker for in vitro teratogenicity screening.

KEYWORDS: apical papilla; CD105; flow cytometry; DART; teratogens.