AP-225

Genome-wide discovery of chromosomal copy number variants in human amniotic cell using array-based comparative genomic hybridization

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Chromosome aberrations (CA) are associated with several genetic heredity diseases. Of concerns, prenatal diagnosis or cytogenetic screening was administered to determine if there are chromosome abnormalities and genetic diseases in a fetus or embryo. However, conventional G-banding technique which is generally applied in CA detection has limit on exploit of copy number variants (CNVs) due to low resolution and tedious multiple processes. Instead, array comparative genomic hybridization (aCGH) technology enabling incremented high-resolution can be considered as an alternative approach for improving prenatal diagnosis. In this study, our aCGH (coverage of SNP tag and CNV regions) was used to identify copy number variations from genomic DNA in human amniotic cell specimens. The aCGH results revealed various karyotypes of CNVs including loss, homozygous loss, gain, high copy gain, and copy neutral LOH whereas using conventional G-banding only one benign cytogenetic CNV was observed in one case study. The aCGH was compatible to define small-scale chromosomal imbalances segment that were undetectable risk segment by G-banding. In contrast, abnormal G-banded karyotypes as balanced rearrangements were hidden from detection by aCGH analysis. The aCGH also provides detailed database of copy number variant regions (CNVRs) in each chromosome. Interestingly, CNVRs containing important genes (ACADM, PPM1B, UGT2B17 and ZDHHC11) were discovered from our data, in which their defects or mutations have been reportedly to be involved in certain genetic heritable diseases and/or syndromes. Gathering together, our detailed CNVs and CNVR via aCGH of amniotic cells might be meaningful database to improve strategy in disease-specific genotoxicity researches.

AP-226

The establishment and validation of androgen receptor mediated stably transfected transcriptional activation assay to detect androgenic and anti-androgenic activities in 22RV1 cells

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The disruption of androgen receptor (AR) mediated androgen signaling played very important roles in several androgen related diseases and symptoms. In order to screen the androgen modulating potency of chemicals, we developed stable AR-GreenS cell lines to access AR mediated transcriptional activation and optimized the protocol. Stable AR-GreenS cell line was stably transfected with pGL4-MMTV/Hygro, which is a firefly luciferase reporter vector bearing androgen responsive element (ARE), in 22RV1 cell line which is a human prostate cancer cells contained functional AR. AR-GreenS cell line was characterized the expression of hormone receptors. In this stable cell line, 5α-Dihydrotestosterone (DHT) was dose-dependently induced the luciferase activity and the activity was significantly increased at 1.0 x 10^{-10} M and stated to reach to plateau 10^{-8} M with maximum about 15 folds compared with vehicle control. This DHT induced luciferase activity was inhibited by the treatment of AR antagonist, bicalutamide. AR-GreenS cells have maintained their growth rate, morphology and responsiveness to DHT until now 85 passages cultured for over 13 months. The inter variation of assay was relatively small with about 5.1± 1.3 mean value of CV (the coefficient of variation). Using AR-GreenS cells, we established the 3 days test protocol and optimized the testing condition for AR transcriptional activation assay. We validated the stable cell line using 20 compounds among 78 substances which are recommended substances for validation of in vitro androgen receptor transcriptional activation assays by ICCVAM.

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