Different Patterns of Cyclin D1/CDK4-E2F-1/4 Pathways in Human Embryo Lung Fibroblasts Treated by Benzo[a]pyrene at Different Doses[1]

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Objective To investigate the roles of the cyclin D1/CDK4 and E2F-1/4 pathways and compare their work patterns in cell cycle changes induced by different doses of B[a]P. **Methods** Human embryo lung fibroblasts (HELFs) were treated with 2 μmol/L or 100 μmol/L B[a]P which were provided with some characteristics of transformed cells (T-HELFs). Cyclin D1, CDK4 and E2F-1/4 expressions were determined by Western blotting. Flow cytometry was used to detect the distribution of cell cycle. **Results** After B[a]P treatment, the proportion of the first gap (G1) phase cells decreased. CDK4 and E2F-4 expression did not change significantly. In 2 μmol/L treated cells, a marked overexpression of cyclin D1 and E2F-1 was observed. However, in T-HELFs overexpression was limited to cyclin D1 only, and no overexpression of E2F-1 was observed. The decreases of G1 phase in response to B[a]P treatment were blocked in antisense cyclin D1 and antisense CDK4 transfected HELFs (A-D1 and A-K4) and T-HELFs (T-A-D1 and T-A-K4). After 2 μmol/L B[a]P treatment, overexpression of E2F-1 was attenuated in A-D1, and E2F-4 expression was decreased significantly in A-K4. In T-A-D1 and T-A-K4, E2F-4 expression was increased significantly, compared with T-HELFs. The E2F-1 expression remained unchanged in T-A-D1 and T-A-K4. **Conclusions** Cyclin D1/CDK4-E2F-1/4 pathways work in different patterns in response to low dose and high dose B[a]P treatment. In HELFs treated with 2 μmol/L B[a]P, cyclin D1 positively regulates the E2F-1 expression while CDK4 negatively regulates the E2F-4 expression; however, in HELFs treated with 100 μmol/L B[a]P, both cyclin D1 and CDK4 negatively regulate the E2F-4 expression.

Key words: Benzo[a]pyrene; Cyclin D1; CDK4; E2F; Cell cycle

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Abbreviations: B[a]P, Benzo[a]pyrene; BPDE, (±)-7β, 8α-dihydroxy-9α,10α-epoxy-7,8,9,10, -tetrahydrobenzo[a]pyrene; CDK4, cyclin-dependent kinase 4; DMSO, dimethyl sulfoxide; DTT, 1,4-dithiothreitol; FBS, fetal bovine serum; HELF, human embryo lung fibroblast; PAH, polycyclic aromatic hydrocarbon; pRb, retinoblastoma protein; SDS, sodium dodecyl sulphate.

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