Polycyclic aromatic hydrocarbons disrupt cell cycle control in rat liver epithelial cells in AhR-dependent manner.

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One of the best known properties of polycyclic aromatic hydrocarbons (PAHs) is activation of aryl hydrocarbon receptor (AhR), a transcription factor which controls expression of a number of genes involved in their metabolism. Recently, several reports have suggested that AhR plays a significant role in cell cycle regulation and PAHs can induce a release from contact inhibition in a model of rat liver immature progenitor cells. This proliferative activity might contribute to carcinogenic properties of PAHs; however its mechanisms are largely unknown. In the present study, we used four PAHs displaying diverse effect on AhR activity and/or proliferation in contact-inhibited WB-F344 rat liver "stem-like" cells: fluoranthene (Fla), a weak activator of AhR with no effect on cell proliferation, benzo[*a*] anthracene (BaA) and benzo[*b*]fluoranthene (BbF) inducing both AhR activation and cell proliferation, and benzo[*a*] pyrene (BaP), a genotoxic AhR ligand inducing apoptosis. We studied their impact on cell cycle regulation and compared them with a model AhR ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Similar to TCDD, BaA and BbF increased cyclin A levels and the activity of cyclin A/cdk2 complex, while expression of cdk2, cdk4, and p27 or pRb phosphorylation were not affected. Fla had no effect on any of the studied proteins. The essential role for cyclinA/cdk2 in BbF-dependent induction of cellular proliferation was confirmed using a cdk2-specific inhibitor roscovitine, which prevented BbF-induced increase in cell numbers. In contrast, BaP induced a significantly higher accumulation of cyclin A and cyclin A/cdk2 activity and decrease in p27 expression, while simultaneously increasing pRb phosphorylation, suggesting that in BaP-treated cells, other cdks are active as well. Thus, genotoxic effects of BaP are associated with both apoptosis and intensive cell proliferation in this model. Using WB-F344 variant stably transfected with a dominant negative AhR mutant, we found that neither BbF nor BaA induced cell proliferation, cyclin A expression or cyclinA/cdk2 activation in dn-AhR cells. Both BaP-dependent apoptosis and increase in cyclin A and cyclin A/cdk2 activity were significantly attenuated in dn-AhR cells. To provide further evidence for the role of AhR in modulation of cyclin A expression, WB-F344 cells were transfected with siRNA targeted against the AhR, which prevented increase in both cyclin A and CYP1A1 expression in BbF-treated cells. In conclusion, AhR plays essential role both in induction of apoptosis and in the release from contact inhibition, which might contribute to tumor promotion. The effects of PAHs are associated with increased cyclin A expression and cdk2 activation. In contrast, strong genotoxin BaP induces both apoptosis and cell proliferation in target cell population, which is also associated with decreased expression of cdk inhibitor p27 and pRb hyperphosphorylation. Attenuation of expression of PAH-activating enzymes in dn-AhR cells is probably responsible for inhibition of apoptosis. [This study was supported by grants No. B6004407 from the Grant Agency of the Academy of Sciences of the Czech Republic and German Research Foundation No. Di 793/1-3.]