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Hugues Jacobs
IGBMC
1 rue Laurent Fries BP 10142
67404 Illikirch
France
Email: hugues@igbmc.u-strasbg.fr

TCDD requires retinoic acid to induce cleft palate

Hugues Jacobs*, Manuel Mark, Norbert B. Ghyselinck. (Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Cnrs/Inserm/Ulp, CU de Strasbourg, Illkirch, France)

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and retinoic acid (RA) exposure in wild type mice at embryonic day (E) 10.5 synergistically induce cleft palate, suggesting a possible interaction between TCDD and RA signalling pathways. Using quantitative RT-PCR, we first showed that neither TCDD nor RA treatments altered expression of genes transducing the RA (i.e., Raldh3, Rarg, Crabp2, Cyp26a1) or AHR (i.e., Ahr, Arnt, Arnt2, Ahrr) signals, respectively. We then analysed palate formation following exposure to TCDD (30microgamme/kg) in Raldh3–/– and Rarg–/– fetuses lacking the RA synthesizing enzyme RALDH3 or the RA receptor RARgamma, respectively. All of them were resistant to cleft palate, indicating the requirement of an intact, active, RA signalling pathway allowing TCDD to induce this malformation. Analysis of reporter activity driven by a RA-responsive transgene in Raldh3–/– palates indicated that RALDH3 is the only provider of RA, while in situ hybridization showed that Raldh3 and Rarg expressions are restricted to nasal epithelium and mesenchyme, respectively. This finding entails that RA synthesized by RALDH3 in nasal epithelium diffuse towards mesenchyme to activate RARgamma, which may control, together with AHR, downstream genes involved in palate formation. Accordingly, we found that Msx1 expression, which is restricted to palatal mesenchyme and whose impairment through gene ablation yields cleft palate, is repressed in RA– or TCDD–treated fetuses. We additionally found that TCDD is less potent to reduce Msx1 expression in Raldh3–/– fetuses. Altogether, our data show that TCDD– and RA–signalling pathways converge to control Msx1 expression, whose impairment likely yields cleft palate.