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Abnormal separation of the respiratory primordium in the adriamycin mouse model of tracheoesophageal malformations

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Abstract

Background/Purpose: Organogenesis relies on temperospatially coordinated signaling systems. The adriamycin rat model provided insights into the dysmorphogenesis of tracheoesophageal malformations. An adriamycin mouse model (AMM) would facilitate the investigation of their molecular pathogenesis. To transfer the knowledge gained from the rat, we describe a histological account of the critical period of organogenesis of these malformations in the AMM.

Method: CBA/Ca mice were accurately time-mated (n = 18). Dams received intraperitoneal injections of adriamycin (6 mg/kg) (n = 12) or saline control (n = 6) on days 7 and 8. Fetuses were harvested on days 9, 9.5, 10, 11, 12, and 13, resin embedded, and 1-μm sections of the developing foregut were examined.

Results: Day 11 control fetuses showed normal separation of the respiratory primordium, with apoptotic bodies at the point of separation. A more caudal point of separation of the distal foregut without apoptotic bodies was found in 4 of 10 AMM fetuses. Day 13 AMM fetuses had dorsal or ventral outpouchings of the foregut, indicating which malformation they would develop. Abnormal branching of the notochord was seen from day 9.5 in AMM fetuses. This was not always associated with abnormal tracheoesophageal development.

Conclusion: This study confirms that the abnormal observations made in the rat model apply to the mouse.

A spectrum of tracheoesophageal malformations occurs in humans. These include esophageal atresia, laryngotracheoesophageal clefts, and tracheal agenesis. These develop with or without a tracheoesophageal fistula. For almost a century, the misconception that the esophagus and trachea develop from an ascending separation of the foregut was used to explain their faulty organogenesis. However, in 1982, Zaw-Tun [1] analyzed early developmental stage human embryos and demonstrated that the respiratory primordium grows caudally from the ventral aspect of the foregut. Tracheoesophageal malformations have been described too rarely in human embryos to be able to detail

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Table 1 The numbers of adriamycin or saline-treated fetuses harvested and examined for each day of gestation

Day of gestation	No. of saline-treated fetuses examined (total harvested)	No. of adriamycin-treated fetuses examined (total harvested)
9	2 (6)	7 (7)
9.5	2 (8)	8 (8)
10	2 (7)	7 (7)
11	2 (6)	10 (10)
12	2 (6)	15 (15)
13	2 (7)	14 (14)

their development [2]. The serendipitous finding that the anthracycline antibiotic adriamycin is teratogenic to rats producing tracheoesophageal malformations has provided a reproducible model in which to gain insights into the dysmorphogenesis of these malformations [3,4]. Because organogenesis relies on temperospatially coordinated signaling systems, the next step is to study the molecular pathogenesis of tracheoesophageal malformations.

The mouse is the foremost mammal studied by developmental biologists, offering an expanding wealth of knowledge and scientific research techniques. Therefore, it is hoped that an adriamycin mouse model (AMM) would better facilitate this process [5]. We have confirmed that the AMM produces a spectrum of tracheoesophageal malformations [6]. To transfer the knowledge gained from the adriamycin rat model (ARM) to the mouse, we describe a detailed histological account of the critical period of organogenesis of these malformations in the AMM.

1. Materials and methods

Eighteen CBA/Ca mouse dams (Harlan UK, Bicester, England), with a mean weight of 23.5 g (range, 16-25 g), were accurately time-mated over a 4-hour period. Identification of a vaginal plug at the end of this mating period was taken to be the start of gestation.

Twelve dams received 2 intraperitoneal injections of adriamycin (Doxorubicin, EBEWE Pharma Ges.m.b.H. Nfg.KG, A-4866 Unterach, Austria) at a dose of 6 mg/kg, 24 hours apart, on days 7 and 8 of gestation. Six dams received intraperitoneal injections of an equivalent volume of a control dose (0.9% sodium chloride) on days 7 and 8 of gestation.

There were 1 control and 2 AMM litters harvested on days 9, 9.5, 10, 11, 12, and 13 (Table 1). Fetuses underwent routine fixation and processing for embedding in Araldite

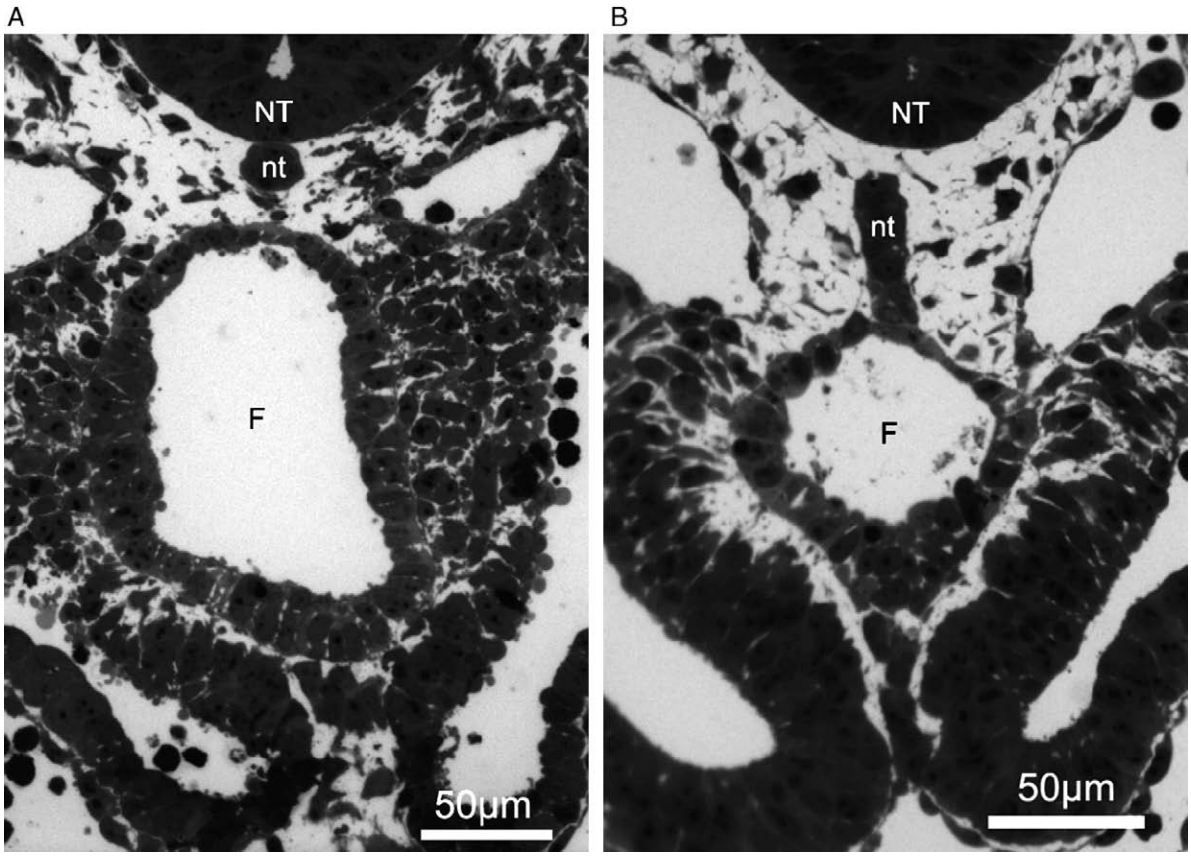

Fig. 1 Transverse sections of the foregut, notochord, and neural tube in a saline-treated (control) fetus (A) and an adriamycin-treated fetus (B) on day 9.5. A, Normal separation of the foregut and notochord has occurred. B, The notochord is attached to a small diameter foregut. F indicates foregut; nt, notochord; NT, neural tube.