Persistent truncus arteriosus in the Splotch mutant mouse

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Summary. The Splotch mutant mouse shows defects in neural crest-derived cell populations. The septation of the truncus arteriosus and the development of the aortic arch-derived blood vessels was studied in homozygotes of the Splotch mutant allele Sp<sup>Tm</sup>. It is shown that in homozygous mutant embryos, the septation of the truncus arteriosus does not proceed normally, resulting in persistent truncus arteriosus. The ostium of the persistent truncus arteriosus opens to the right ventricle. Frequently, variations of the aortic arch-derived blood vessels are observed. The development of the thymus, the parathyroid and the ultimobranchial bodies are also variably affected in mutants.

These results provide indirect evidence, that cells contributing to the aortic arches and the septum of the truncus arteriosus in mice are derived from the neural crest. The Splotch mutant mouse is proposed to be an animal model for persistent truncus arteriosus. The implications of the vascular malformations for the midgestational death of this mutant are discussed.

Key words: Mouse – Blood vessels – Neural crest – Aortic arches

Introduction

In mice, the formation of the major blood vessels, which supply the upper extremities and the head is completed by the thirteenth day of gestation. The third, fourth and sixth aortic arches and the ventral and dorsal aortic root, are remodelled to give rise to the subclavian, common carotid, and pulmonary arteries, the ductus arteriosus Botalli and a segment of the adult aortic arch. The single outflow vessel of the developing heart, the truncus arteriosus (TA), which connects the heart with the aortic arches, is divided by a septum, that grows from the aortic arches towards the primitive valve of the TA. The truncal septum together with the aortico-pulmonary septum divide the cardiac outflow tract, thus forming the ascending aorta and the pulmonary trunk, which connect to the left resp. right ventricle (Theiler 1972). Cell fate studies in birds, involving chick/quail chimeras, have shown that the neural crest gives rise to cells forming the truncal and the aortico-pulmonary septum (Kirby et al. 1983; Phillips et al. 1987) and also contributes to the walls of the major aortic arch-derived blood vessels (LeDouarin 1982). The importance of these contributions in birds was corroborated by others, who showed that ablation of the “cardiac neural crest” induces malformations of the ventricular outflow and abrogates the septation of the truncus arteriosus, thus causing persistent truncus arteriosus (PTA) (Nishibatake et al. 1987; Besson et al. 1986).

Studies of PTA have most extensively been performed in avian embryos, because these are more easily accessible to manipulations during embryogenesis. Similar manipulations would be difficult to do in mammalian embryos because of their limited survival outside the uterus. Malformations caused by mutagens, e.g. Win 18,446-induced PTA in rats (Taleporos et al. 1978), are useful to obtain a given phenotype, but genetically defined mutants might prove more promising for the subsequent analysis of the molecular and cellular basis of the developmental step concerned.

Splotch (Sp<sup>T</sup>) is a semidominant locus on chromosome 1 of the mouse (Russell 1947). Heterozygous mutants of the Splotch locus (Sp<sup>T</sup>+) show white spotting on the belly, the tail and the feet. Homozygotes (Sp<sup>T</sup>/Sp<sup>T</sup>), however, can be recognized on day 10 of gestation because of the failure of the “cardiac neural crest” induces malformations of the ventricular outflow and abrogates the septation of the truncus arteriosus, thus causing persistent truncus arteriosus (PTA) (Nishibatake et al. 1987; Besson et al. 1986).

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Fig. 1. Homozygous mutant embryo (Sp<sup>Tm</sup>/Sp<sup>Tm</sup>) on day 13.5 of gestation, showing rachischisis and cranioschisis. Note the abnormal kink of the tail. The fore-footplates show indentations, but not yet individual phalanges, the latter being an external characteristic feature of day 14 of gestation.
of the neural tube to close in the lumbo-sacral region and in 50% of the cases also in the region of the prospective hind brain (Auerbach 1954). Live homozygotes are not found beyond day 13 of development (day of vaginal plug being day 1), while heterozygotes and wildtype littermates cannot be distinguished macroscopically at this stage of development (Auerbach 1954).

In a detailed histological analysis of these mutants, Auerbach (1954) showed that the defect of neurulation in Splotch homozygotes is associated with a dysgenesis or agenesis of neural crest-derived tissues such as spinal ganglia and the pigment cells of the skin. Moreover, an electron microscopic examination of the neural crest-derived Schwann cells on motoric nerves of homozygous Splotch mutants showed that they do not lay down a basal lamina in thoracic segments and are not formed at all in lumbar areas of the developing embryo (Franz 1989).

In this report, the development of the aortic arches and the septation of the truncus arteriosus as well as the thymus, parathyroid, thyroid glands and the ulemobranchial bodies were investigated in homozygous Sp<sup>TM</sup> mutant embryos at mid-gestation. It is shown that the Sp<sup>TM</sup> allele interferes with the normal septation of the TA in conjunction with deviations from the normal development of the pharyngeal pouch-derived glands.

**Materials and methods**

**Mice.** Sp<sup>TM</sup> is a radiation-induced allele of the Splotch locus (Beechey and Searle 1986). Sp<sup>TM</sup>/+ males in a (C3H x 101)F<sub>1</sub> background were obtained from MRC Radiobiology Unit Harwell, Didcot, England. These were crossed with C57BL/6 females obtained from Institut für Versuchstierzüchtung, Hannover, Federal Republic of Germany. Heterozygotes (Sp<sup>TM</sup>/+) among the F<sub>1</sub> generation of these crosses were identified by the presence of a white belly spot. All animals were maintained at a 12 h light/dark cycle with food and water ad lib. To obtain homozygous mutants, heterozygous females in estrous were caged with heterozygous males in the evening and were examined for the presence of a vaginal plug in the morning. Noon of the day, on which the plug was detected, was termed day 0.5 of gestation.

**Dissection of embryos.** Pregnant females were killed by cervical dislocation. The embryos were dissected from the uterus in a Petri dish filled with PBS. The yolk sac and amnion were opened and the umbilical cord cut. The pulsating stream of blood indicated ongoing contractions of the heart in live embryos. Embryos destined for histological examinations were then immersed in fixative.

**Histology.** Embryos dissected from the yolk sac and amnion were fixed by immersion in Carnoy's solution for three hours, dehydrated in absolute ethanol for one hour and kept in methylbenzoate overnight, and subsequently embedded in paraffin. Sections of 5 μm thickness were obtained on a Jung microtome, dried and deparaffinized in Xyloil. Slides were run through a descending alcohol series and stained with hematoxylin/eosin. After staining, slides were dehydrated in a ascending alcohol series and mounted in Eukitt.

**Results**

**Segregation of the Sp<sup>1H</sup> allele**

Midgestational embryos of Sp<sup>1H/+</sup> × Sp<sup>1H/+</sup> crosses were dissected and the number of homozygous mutants determined according to the presence of rachischisis and/or cranioschisis. Examination of 13 litters between days 10 and 14 of development showed that 28.4% of the embryos were phenotypically Sp<sup>1H</sup>/Sp<sup>1H</sup> (Table 1). Some 6.5% of the implants had been resorbed on the day of dissection and could no longer be classified.

The examination of litters on days 13.5 and 14.5 of gestation respectively revealed that of 10 homozygotes detected on day 13.5 of gestation all were alive, showing heartbeat, blood circulation and apart from their pathognomonic features no obvious developmental retardation (Fig. 1). All six homozygotes detected on day 14.5 of gestation, however, were dead and being resorbed. These homozygotes must have died between day 13.5 and day 14 of gestation, because they were somewhat smaller than their phenotypically normal littermates. Moreover, they still showed paddle-shaped fore-footplates, while the feet of their normal littermates had already developed individual phalanges, which is the external, characteristic feature for this step in development (Theiler 1972).

Outcrosses of Sp<sup>1H</sup>/+ (C3H x 101)F<sub>1</sub> males to wildtype C57BL/6 females showed that 41.8% of the F<sub>1</sub> generation (n = 246) inherited the white belly spot characteristic of Splotch heterozygotes (Table 1). The number of observed heterozygotes is significantly different from the expected 50% (p < 0.01), which may indicate that the Sp<sup>1H</sup> dominant phenotype is not fully penetrant or that some heterozygotes are lost during gestation.

These results demonstrate that the Sp<sup>1H</sup> allele of the

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<th>Table 1. Segregation of the Sp&lt;sup&gt;1H&lt;/sup&gt; allele in: I. outcrosses of Sp&lt;sup&gt;1H&lt;/sup&gt;/+ males to C57BL/6 females and II. intercrosses of Sp&lt;sup&gt;1H&lt;/sup&gt;/+ mice</th>
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<sup>a</sup> Embryos that could no longer be classified because of resorption

<sup>b</sup> The discrepancy between the observed 41.8% and the expected 50% is significant, p < 0.01

<sup>c</sup> Wildtype and heterozygote embryos were combined, since they are morphologically indistinguishable

<sup>d</sup> This represents the sum of 14.6% animals with Spina bifida alone, and 13.8% animals with a combination of Spina bifida and cranioschisis