MORPHOGENESIS OF AGGREGATES FORMED ON RECOMBINATION OF LUNG EPITHELIUM AND MESENCHYME OF INTACT AND URETHANE-TREATED MOUSE EMBRYOS

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Intercellular and intertissue interactions are known to play a definite role in normal embryogenesis and organogenesis [2]. The transplacental action of chemical carcinogens on the developing organism causes the formation of malignant neoplasms in the progeny [7-9]. It can be postulated that disturbance of epithelial–mesenchymal interactions in embryonic target organs is of great importance in the mechanisms of transplacental carcinogenesis. To investigate this problem epithelial–mesenchymal interactions were studied during transplacental lung carcinogenesis. Epithelial–mesenchymal recombinations of the target organ in intact and experimental animal embryos were used as the model.

It was shown previously that dissociated cells of normal embryonic lungs form organotypical aggregates under certain conditions, which are capable of growth and differentiation during long-term culture [1, 4-6]. This paper gives the results of a comparative study of the morphogenesis of long-term cultures of aggregates obtained by recombination of epithelium and mesenchyme of the lungs of intact and experimental mouse embryos.

EXPERIMENTAL METHOD

Lungs of 17-18 day line A mouse embryos were used. Urethane was injected subcutaneously, in 10% physiological saline, into the mothers on the 15th and 16th days of pregnancy, in two injections each of 1 g/kg body weight. To separate the epithelium and mesenchyme of the embryonic lungs, a method devised by the writers was used: It consisted of a combination of enzymic and mechanical treatment of the minced organ followed by fractional precipitation of epithelial complexes from a suspension of single mesenchymal cells [5]. To obtain organotypical aggregates the following epithelial–mesenchymal recombinations of the separated tissue components of lungs of intact and experimental embryos were used: 1) Epithelium and mesenchyme were taken from intact (control) embryos (EcMc); epithelium was taken from intact and mesenchyme from experimental embryos (EcMe); 3) epithelium was taken from experimental and mesenchyme from intact embryos (EeMc); 4) epithelium and mesenchyme were both taken from experimental

TABLE 1. Frequency of Hyperplastic and Metaplastic Changes in Epithelium in Cultures of Aggregates Formed by Recombination of Tissue Components of Embryonic Lungs from Intact and Urethane-Treated Mice

Type of aggregate	No. of aggregates (total)	Hyperplasia of epithelium			Squamous-cell metaplasia of epithelium		
		No. of aggregates		P	No. of aggregates		P
		absolute	%		absolute	%	
EcMc	75	3	4,0	—	—	—	—
EcMe	66	17	25,7	$P_1<0.001$	1	1,5	$P_1>0,1$
EeMc	65	17	26,1	$P_1<0,001$	1	1,5	$P_1>0,1$
EeMe	71	21	29,6	$P_1<0,001$ $P_2>0.1$	7	9,9	$P_1<0,01$ $P_2<0,1$

Legend. P_1) compared with EcMe, P_2) compared with EcMe and EeMc.

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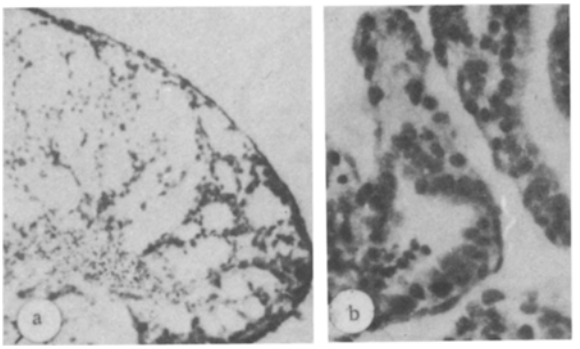

Fig. 1

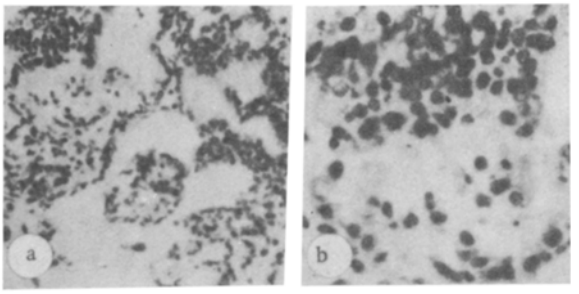

Fig. 2

Fig. 1. Growth and differentiation of organotypical tissue structures of aggregates of EcMc type, obtained from epithelium and mesenchyme of lungs of intact control mouse embryos. a) Alveolus-like structures, 7 days of culture, 200 ×; b) branching bronchial structures lined with cylindrical epithelium, 21 days of culture, 500 ×.

Fig. 2. Hyperplasia of epithelium. a) Aggregates of EeMe type, 21 days of culture, 200 ×; b) the same, 500 ×; c) aggregate of EcMe type, 14 days of culture 500 ×.

[Letters for Figs. 2 and 3 as in Russian original; presumably their captions were inadvertently switched — Publisher.]

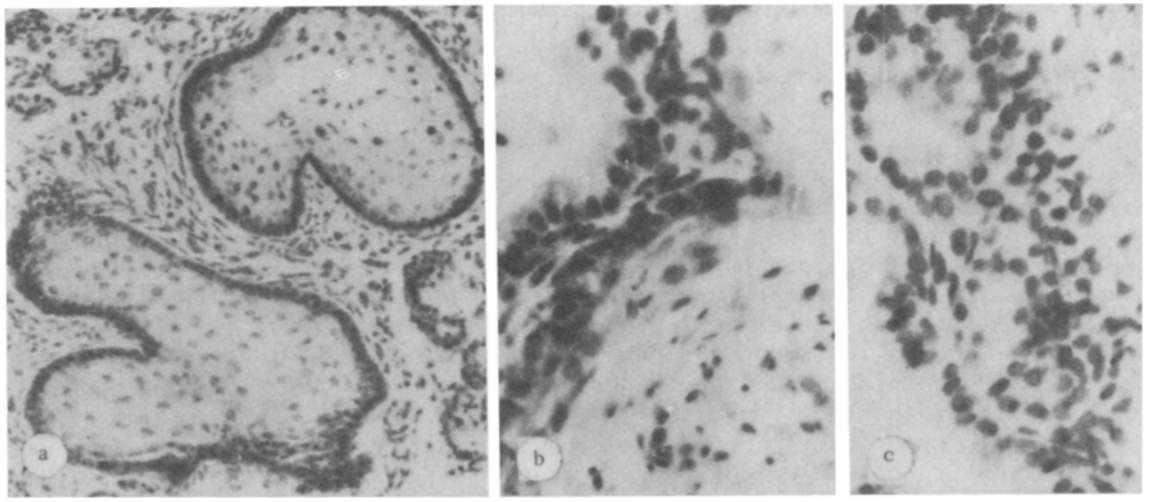

Fig. 3. Squamous-cell metaplasia of epithelium. a) Aggregate of EeMe type, 7 days of culture; cells of squamous epithelium completely filled lumen of bronchii, 200 ×; b) aggregate of EeMc type, 21 days of culture; keratinizing squamous epithelial cells can be seen, 500 ×.

embryos (EeMe). Mixed suspensions of the corresponding fractions of mesenchymal and epithelial cells were prepared by the method in [5] and cultured by a modified hanging drop method [6]. The formed aggregates were then transferred to the surface of millipore filters and culture continued by the organ culture method [1]. After 4, 7, 14, and 21 days of the experiment the aggregates were fixed with Bouin's fluid and, after histological treatment, they were examined with the light microscope in serial sections stained with hematoxylin and eosin. The results were subjected to statistical analysis by the chi-square method.

EXPERIMENTAL RESULTS

After 4 days of culture the formed aggregates obtained by recombinations of epithelium and mesenchyme of embryonic lungs of intact and experimental mice as described above had a similar organotypical structure, which was indistinguishable from that of aggregates studied

previously and formed from dissociated embryonic lung cells of intact animals, not separated into tissue components [4, 6]. Their tissue consisted of a large number of alveolus-like "air" cavities lined with cubical epithelium, with single elongated mesenchymal cells adjacent to their basement membrane. The surface of the aggregates was covered with a layer of mesenchymal fibroblast-like cells.

Starting with the 7th day of culture differences appeared in the morphological structure of the different types of aggregates. Further growth and differentiation of organotypical alveolus-like and bronchiol-like structures (Fig. 1) were observed. Only in a few aggregates (4.0%) were trivial hyperplastic changes found in the epithelium. The dynamics of morphogenesis of such aggregates was described in detail preiously [ ]. Marked hyperplastic and metaplastic changes in the epithelium were found in culture of aggregates obtained by recombination of tissue components of the lungs of experimental embryo (EeMe) and also of intact and experimental embryos (EcMe). Small foci of hyperplastic proliferation of the epithelium (Fig. 2), in which polymorphism and atypism of the cells and mitoses were observed as a rule, were found most frequently. Squamous-cell metaplasis of the bronchial epithelium was accompanied in most cases by keratinization of the cells and hyperplastic proliferation of the basal epithelium (Fig. 3). Hyperplastic changes in the epithelium also were seen in the surrounding tissue.

The frequency of areas of hyperplastic proliferation was virtually the same in aggregates in which both components or only one of them were taken from the lungs of experimental mouse embryos (Table 1). Squamous-cell metaplasia of the epithelium was found in significant frequency only in aggregates in which both tissue components were taken from experimental embryos (EeMe).

Hyperplastic proliferation of the epithelium was similar in its morphology to that observed previously in adenoma formation induced in organ cultures of embryonic mouse lungs exposed to the transplacental action of urethane [3, 7]. It is important to note that these hyperplastic changes, which are the early stages of development of adenomas of the lung, appeared with sufficiently high and equal frequency in all types of aggregates mentioned above (EeMe, EeMc, and EcMe). This is evidence that "experimental" mesenchyme can induce hyperplastic proliferation of "intact" epithelium on contact and by morphogenetic interaction, and that "experimental" epithelium, despite contact and interaction with "intact" mesenchyme, preserves its ability to undergo hyperplastic proliferation induced by the transplacental effect of urethane. As regards squamous cell metaplasia of the epithelium, by contrast with hyperplasia, it was not found even once previously in a study of transplacental formation of adenomas of the lungs in experiments both *in vivo* and *in vitro* [3, 7]. Squamous-cell metaplasia of the epithelium arising in the aggregates was evidently the result of disturbances of intertissue morphogenetic interaction, caused by urethane and demonstrable in this particular model. It will be noted that the frequency of metaplastic changes was many times higher in those aggregates in which both tissue components were exposed to the action of urethane.

These results are thus evidence of the importance of disturbance of epithelial–mesenchymal interrelations in the mechanisms of transplacental lung carcinogenesis. The results of a study of morphogenesis of different types of aggregates confirmed the previous hypothesis on the important role of the mesenchyme of the embryonic lungs in the realization of the transplacental effects of pulmonotropic carcinogens on epithelial target cells [5], effected through subsequent intertissue epithelial–mesenchymal interactions.

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