Specific Spindle Cells and Certain Mesenchymal Cells in Tooth Germ

By

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Introduction

In the previous study entitled "Specific Spindle Cells and Globular Substances in Enamel Knot" the author reported that (1) the specific spindle cells appear at the free ends of the tooth bud and in the enamel knot, and they have a specific stain affinity and the characters of their staining powers are not nucleic-acid, and (2) certain irregular-shaped mesenchymal cells, whose nuclei showed almost the same hue as that of the spindle cells with Masson Goldner stain and Mallory Heidenhain stain, were present in the dental papilla.

Since there was no previous report on these structures in the tooth germ, the author investigated the nucleoprotein of these cells histochemically, and these results will be reported in this paper.

Materials and Methods

Eight pregnant female DDD strain’s mice were used. The mice were killed on the 11th day to 14th day after conception. The fetal heads, 52 in total, were prepared for histochemical methods. The fixation, embedding, thickness of the frontal serial section and histochemical method are as follows:

<table>
<thead>
<tr>
<th>Fixation</th>
<th>Embedding</th>
<th>Thickness of sections</th>
<th>Histochemical methods</th>
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<tbody>
<tr>
<td>Carnoy</td>
<td>Paraffin</td>
<td>5μ</td>
<td>1) Naphthol yellow S reaction</td>
</tr>
<tr>
<td>10% neutral formalin</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2) Millon reaction</td>
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<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>3) Fast green FCF reaction</td>
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<td>4) DMAB reaction</td>
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They were processed according to the technique of the original works for the histochemical detection of nucleoprotein.
Results

1) Naphthol yellow S reaction (Fig. 1)

The spindle cells in the dental epithelium presented two kinds of reactions: (1) Within the nuclei except for the nucleolus no reaction was recognized (—) and the nucleo-membranes showed a brown colour, or (2) the nuclei reacted markedly with the Naphthol yellow S reaction and became deep yellow (++).

The dental epithelial cells other than the spindle cells were positive (+), but the differences among them were not clear.

Some of the irregular-shaped mesenchymal cells in the dental papilla (including the future dental papilla), whose nuclei showed the same reaction as the spindle cells were recognized. The nuclei of the other mesenchymal cells were positive with Naphthol Yellow S reaction (+).

2) Millon reaction

The nuclei of the spindle cells and certain irregular-shaped mesenchymal cells did not show any reaction (—), but the dental epithelial cells other than the spindle cells and the nuclei of the mesenchymal cells other than certain irregular-shaped mesenchymal cells showed a faint pink colour (±).

3) Fast Green FCF reaction (Fig. 2, Fig. 3)

The spindle cells showed two kinds of reactions. (1) The nuclear components other than the nucleolus did not react with the Fast Green FCF reaction (—), or (2) they became conspicuously deep green (++). The nuclei of the cells other than the spindle cells in the enamel organ were positive (+).

Some of the irregular-shaped mesenchymal cells showed almost

<table>
<thead>
<tr>
<th>Histochemical Method</th>
<th>the nuclei of the spindle cells</th>
<th>the nuclei of other dental epithelial cells than spindle cells</th>
<th>the nuclei of certain irregular-shaped mesenchymal cells</th>
<th>the nuclei of other mesenchymal cells than certain irregular-shaped cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthol Yellow S reaction</td>
<td>++ or —</td>
<td>+</td>
<td>++ or —</td>
<td>(+)</td>
</tr>
<tr>
<td>Millon reaction</td>
<td>—</td>
<td>—</td>
<td>±</td>
<td>(±)</td>
</tr>
<tr>
<td>Fast Green FCF reaction</td>
<td>++ or —</td>
<td>+</td>
<td>++ or —</td>
<td>+</td>
</tr>
<tr>
<td>DMAB reaction</td>
<td>—</td>
<td>±</td>
<td>—</td>
<td>±</td>
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</tbody>
</table>
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the same reaction as the spindle cells in the dental papilla (including the future dental papilla), and the other mesenchymal cells were positive with the Fast Green FCF reaction.

4) DMAB reaction

The nuclei of the spindle cells were negative with the DMAB reaction (−), but the other dental epithelial cells reacted to some extent with it (+). In the outer enamel epithelium (including the future outer enamel epithelium), the cytoplasm was positive at the basal and apical ends (++) . In the dental papilla (including the future dental papilla) the nuclei of certain irregular-shaped mesenchymal cells did not react in the least, and the nuclei of the other mesenchymal cells scarcely reacted.

Discussion

It is said that the Naphthol Yellow S reaction and Fast Green FCF reaction stain the basic nucleoprotein (Deith 1955, Alfert 1953). Millon reaction stain the tyrosin pink red, rose, and red histochimically (Bensley and Gersh 1933). The DMAB reaction stains the tryptophan blue (Adams 1957).

The spindle cells and certain irregular-shaped mesenchymal cells showed the following reactions with the Naphthol Yellow S reaction. Within the nuclei, except for the nucleolus, no reaction was recognized, or the nuclei of these cells reacted more markedly than the other cells in the tooth germ. With Millon reaction and the DMAB reaction they were negative.

In some case, except in the nucleolus in the spindle cells and some irregular mesenchymal cells, the basic nucleoprotein was not present. These facts seem to suggest that basic nucleoprotein-lysis had occurred. In other cases, the nuclei of these cells were stained markedly green with the Fast Green FCF reaction and yellow with the Naphthol Yellow S reaction. Leuchtenberger (1950) states that in the process of the pycnotic changes it seems there is an increase of the basophile components in the nuclei, that in fact it is nothing but the concentration of the nucleoprotein quantitatively. It is reasonable to conclude from these facts that the spindle cells in the dental epithelium and some irregular-shaped mesenchymal cells in the dental papilla are degenerated or degenerating cells. It is thought that these cells show physiological regressive changes and this phenomenon belongs to “Necrobiosis” as described by De Robertis, Nowinsky, and Saez (1965). The significance of these cells is a matter of grave concern. In the tooth germ, they appear at a peculiar stage and place, that is to say, they are present at the free end of the tooth.
bud, in the enamel knot and in the surrounding parts of it, and in the dental papilla (including the future dental papilla) (Nozue 1971). The enamel knot is considered as the place where the cusp will be formed in the future (Reichenbach, 1926; Santone, 1935; Butler, 1956; Gaunt and Miles, 1967). It is said that the basic proteins in the nuclei control the gene group. It is assumed that these cells have something to do with the morphogenesis of the tooth. Lately it was reported that in the tissue culture of the tooth germ, the development of the epithelium-like cells was observed first, followed by the migration of the fibroblast-like cells (Borea and Castaldini 1969). De Beer (1947) states that the dental papilla in the Amblystoma is derived from the neural crest cells. Miles and Gaunt (1967) state that from the findings by Pourtois (1961) combined with those of Dalq and Mulnard (1953) and Milaire (1959), it is quite clear that within the head of the very early mouse embryo widespread cell migrations take place, particularly associated with the neural-crest derived ectomesenchyme. The origin of the spindle cells in the dental epithelium is interesting. Further studies into this problem will throw light on the morphogenesis of the tooth.

The dental epithelial cells other than the spindle cells and the mesenchymal cells other than some irregular-shaped mesenchymal cells showed the faint colour with the Millon and the DMAB reaction. It is reasonable to presume that these results do not show an active reaction. Protamin is not composed of tyrosin or tryptophan, but arginin (Bloch and Hew 1960). It is said that histon is positive with the Millon reaction, and histon and protamin are distinguished by the Millon reaction (Folin and Ciocalten 1927). The nucleoprotein of the tooth germinal cells other the spindle cells and certain irregular shaped mesenchymal cells is thought to be composed not chiefly of the histon, but of the protamin.

**Summary**

(1) The nuclei of the spindle cells in the enamel organ and certain irregular-shaped mesenchymal cells in the dental papilla (including the future dental papilla) showed the following reaction. Except for the nucleolus they did not react with the Naphthol Yellow S reaction and Fast Green FCF reaction, or they showed a more marked reaction than the nuclei of the other tooth germical cells with the above method. They were negative with the DMAB reaction and the Millon reaction.

(2) The above results suggest nucleoprotein-lysis or concentration of the nucleoprotein. These cells seem to be degenerated or
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degenerating cells.

(3) The nucleoprotein of the tooth germinal cells other than the spindle cells and certain irregular-shaped mesenchymal cells is thought to be composed not chiefy of the histon, but of the protamin.

(4) The significance of these cells remains obscure, and further experimental study on the origin of these cells will throw light on the morphogenesis of the tooth.

References


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633-656, 754-767, 1935.

**Key to Illustration**

**abbreviations**

D: Dental epithelium  
IR: Certain irregular-shaped mesenchymal cells  
M: Mesenchyme (include the dental papilla)  
SP: Spindle cell