ELECTRON MICROSCOPIC STUDY ON VON RECKLINGHAUSEN'S DISEASE

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SUMMARY: Three cases of von Recklinghausen's disease were examined electron-microscopically to obtain characteristics of cellular arrangement and of component cells in the tumors. The tumors consisted of the axons, Schwann cells and fibroblasts, associated with abundant collagenous fibers. Three types of Schwann cell proliferation were found; 1) almost normal structure of the peripheral nerve with axon, 2) Schwann cell wrapping itself in its cytoplasmic process without axon, 3) discrete Schwann cell with neither axon nor wrapping cytoplasmic process. Each Schwann cell element was invariably provided with basement membrane and the axons were all unmyelinated. Dense areas like desmosomes were scarcely present between the wrapping cytoplasmic processes. The fibroblasts were closely connected with the Schwann cells, being present in the gap of wrapping Schwann cell or in contact with the outside of wrapping one. The Schwann cell was capable of producing collagenous fibers, although the majority of fibers in the tumors would be derived from the fibroblasts. Also, the Schwann cell was observed to produce the fibers showing the peculiar banded structure at the end of wrapping cytoplasmic process, which was the same area as the collagenous fibers were formed.

INTRODUCTION

Von Recklinghausen’s disease (multiple neurofibromatosis) is known as a heredofamilial disseminated phacomatosis with tumors of neurofibroma type which is histologically characterized by circumscribed and non-encapsulated proliferation of the peripheral nerve elements. On electron microscopy, Takahama (1963) already described the neurofibroma as isolated Schwann cell elements intermingled with fibroblasts in an abundant collagenous matrix. There has been controversy as to whether collagen in the tumor could be produced by the Schwann cell and/or the fibroblast. Nathaniel and Pease (1963) presented an evidence of collagen production by the Schwann cells on the basis of an electron microscopic study on events after nerve injury in rats. Others (Thomas, 1964; Hilding and House, 1965) confirmed this observation.

On the other hand, Thomas (1964) pointed out that basement membrane formation was typical of the Schwann cells, by which they were distinguished from the fibroblasts. Pillai (1964) reported “a banded structure” in the experimentally constricted part of the nerve. This peculiar structure to the Schwann cell was found not only in neurofibromas, but also in neurilemmomas and brain tumors (Thomas, 1964; Hilding and House, 1965).
We recently examined electron-microscopically the specimens of nodules of the skin removed from three patients of von Recklinghausen's disease. A variety of features were obtained, from a proliferation revealing an almost normal structure of the peripheral nerve to that of Schwann cell alone. Details of the banded structure, collagenous fiber and basement membrane formations of the Schwann cells observed in the features will be described in this paper.

**MATERIALS AND METHODS**

Three cases histologically diagnosed as neurofibroma were examined.

*Case 1:* Multiple tumors in the trunk and extremities of a 32-year-old female.

*Case 2:* Sporadic tumors in the lower abdomen and bilateral inguinal region of a 75-year-old male.

*Case 3:* Sporadic tumors in the trunk and extremities of a 65-year-old female.

Specimens of case 1, 2 and 3 were removed from tumors of her back, of his left inguinal region, and of her right forearm, respectively.

The specimens were fixed in standard 1% buffered OsO₄ solution added with 0.045 gm/ml sucrose, embedded in Epon and sectioned with a LKB-microtome. The sections were stained with lead citrate and examined with an AKASHI-80 type electron microscope. The Epon blocks were trimmed under control of preliminary sections examined with a light microscope after toluidine blue staining.

**RESULTS**

Tumors examined were found in the superficial subcutaneous tissues and some tumor sites rose above the skin level. The tumor consisted of Schwann cells, fibroblasts and fairly abundant collagenous fibers. Both cells were intermingled and loosely arranged to show no particular pattern. The bundles of fiber stretched to various directions in general. The Schwann cell was invariably provided with basement membrane while the fibroblast was not. The basement membrane was shown as a hazy, homogeneous layer about 500 Å thick.

The axons shown in the pictures (Figs. 1, 2 and 3) were all unmyelinated. The groups of unmyelinated axons were able to distinguish, each group being associated with a different Schwann cell. The groups of axons were further folded and refolded by the proliferation of Schwann cell. These features seem to reproduce an almost normal structure of the peripheral nerve. Even in the absence of axon, the characteristic of folding and refolding was displayed (Fig. 4). The Schwann cell wrapped itself in its own cytoplasmic process. Infrequently, a discrete Schwann cell did not reveal such characteristic but its cytoplasmic process stretched straight (Fig. 5).

From the above findings on the Schwann cell proliferation, the following three types are indicated; 1) almost normal structure of the peripheral nerve with axon (Figs. 1, 2 and 3), 2) Schwann cell wrapping itself in its cytoplasmic process without axon (Fig. 4), and 3) discrete Schwann cell with neither axon nor wrapping cytoplasmic process (Fig. 5).

The dense areas (Fig. 3), similar to desmosome, were observed between the wrapping cytoplasmic processes being in contact with each other.

The cytoplasm of Schwann cell contained a number of fibrils and microtubular structures at a distance from the nucleus (Fig. 6). There were some vesicles and small
mitochondria in the cytoplasm. The ribosome and the endoplasmic reticulum were commonly scanty in contrast with those of the fibroblast.

The fibroblasts were found to be closely connected with the Schwann cell. They were seen not only in contact with the outside of wrapping Schwann cell (Fig. 4), but thin cytoplasmic processes of them were also seen in the gap of wrapping Schwann cell (Figs. 1 and 3). They are likely thought to be a derivative of end- or perineurium as a component of the peripheral nerve.

Although the majority of the collagenous fibers in the tumor were considered to be derived from the fibroblasts, it was recognized that the Schwann cell can also produce the fibers. Numerous collagenous fibers were present adjacent to the Schwann cell and were apparently in contact with the end of the elongated cytoplasm (Fig. 1). Figure 6 represents a high magnification view of the similar finding. The fibers directly contact with the ends of the cytoplasm, where there are neither distinct cell membrane nor basement membrane. It is therefore likely that collagen is being formed in these areas of the Schwann cell.

A peculiar banded structure is seen in the same area as the collagenous fibers are formed in Fig. 1. An insert in Fig. 1 shows a high magnification of the structure, diameter of each fibril being approximately 50 Å with periodicity of about 1500 Å. The banded structure is emerging from the end of cytoplasmic process of Schwann cell without intervening basement membrane or cell membrane. The upper outer part of it is slightly piled on the collagenous fibers and a few banded fibrils stretch along the cell surface covered with the basement membrane. The picture illustrated is a typical area, in which the banded structure is presumably being formed. The banded structure is shown as another example in the center of Fig. 2, which is spindle in shape and directly intermingled with the collagenous fibers.

DISCUSSION

At optical level the feature of the tumor is rather monotonous and non-characteristic as indicated in Fig. 7. It is hard to distinguish between two kinds of the component cells by the optical observation. However, the electron microscopy reveals every characteristic of Schwann cell and fibroblast as pointed out by Oota and Takahama (1962). On the electron microscopic observation, both the components and their arrangement mimic a normal peripheral nerve, in other word, the Schwann cell proliferates preserving a character of wrapping in connection with the fibroblastic element.

Re-examining the specimen at the optical level, we can now recognize a similar cellular arrangement that the tumor cell is enfolding another cell, as if it were a cut surface of capillary (Fig. 7).

The collagenous fibers derived from the Schwann cells may be minor in number. It is most likely that in the Schwann cells, collagen can mainly be produced at the end of the elongated cytoplasm internal to the folding cytoplasmic process as indicated in Figs. 1 and 6. The fibroblastic cells found to be discrete or in contact with the outside of wrapping Schwann cell (Figs. 5 and 4) would possibly produce the majority of fibers in the tumors. Incidentally we may remark that since Stout and Murray (1942) showed that the Schwann cells can produce the connective tissue fibers in tissue culture studies, it has been believed that the endoneurium and perhaps the perineurium are formed by the Schwann cells (Stout, 1949). However, our observations incline us to support the classical view that they are basically fibrous mesodermal origin.
In addition to the fibrogenesis, it is of interest to note that the banded structure which was observed by Takahama (1963) is found in the similar area as collagen is produced (Figs. 1 and 2). This finding indicates that the banded structure is closely related to collagen production. The banded structure has so far been found in traumatized nerve (Pillai, 1964), neurinoma (Takahama, 1963; Wechsler and Hossman, 1965), and neoplastic neuroma (Hilding and House, 1965). From these facts, it is inferred that it can be induced by abnormal Schwann cell being hyperplastic or neoplastic, not by normal one (Luse, 1960). Under the special condition of tissue culture, fibroblasts were also recognized to produce a similar banded structure (Goldberg and Green, 1964).

The axons we could find were unmyelinated without exception. Therefore, the tumors of Recklinghausen's disease may be derived from a sensory fiber from the spinal ganglia or a post-ganglionic sympathetic fiber.

Assuming that the three types of Schwann cell proliferation reflect developmental process of the tumor, type 1 is considered to be an original structure, in which the axons had possibly existed before the tumor developed. Some of the proliferated Schwann cells would detach from the original structure and become discrete, namely type 3. As the discrete Schwann cell matures, it would reveal the character of wrapping indicated as type 2. The type 2 is most frequently found in the tumors and the other two types are rare.

In the present study, we did not obtain any causative agent responsible for the Schwann cell proliferation.

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REFERENCES


LEGENDS FOR FIGURES

Key:  
Ax=Axon  
BM=Basement Membrane  
BS=Banded Structure  
CF=Collagenous Fiber

D=Dense area like desmosome  
Fib=Fibroblast  
Sch=Schwann Cell

With the exception of Fig. 7, which is a photomicrograph, all the figures are electron micrographs. Horizontal line represents 1 μ.

Fig. 1: Schwann cell proliferation reproducing almost normal structure of peripheral nerve with axons. Note formation of both fibers, one is collagenous fibers and the other is peculiar fibers showing banded structure, at the ends of cytoplasmic processes of Schwann cells. Slender cytoplasm of fibroblast, which is not covered with basement membrane, is shown inside the wrapping Schwann cell.

Insert: Enlargement of the boxed area, in which horizontal line represents 0.5 μ. Banded structure, of which upper outer part is slightly piled on collagenous fiber, is emerging from the end of cytoplasm.
Fig 2: Schwann cell proliferation reproducing almost normal structure of peripheral nerve with axons. Note formation of banded structure, which directly intermingles with collagenous fibers.
Fig. 3: Schwann cell proliferation reproducing almost normal structure of peripheral nerve with axons. Slender cytoplasm of fibroblast, which is not covered with basement membrane, is shown inside the wrapping Schwann cell. Dense areas like desmosome are present between the wrapping cytoplasmic processes of Schwann cell.

Fig. 4: Schwann cell wrapping itself in its cytoplasmic process, not associated with axon. Fibroblasts are in contact with outside of wrapping Schwann cell.
Fig. 5: Discrete Schwann cell and fibroblast. The former is provided with basement membrane and the latter is not. The cytoplasmic process of Schwann cell stretches straight.

Fig. 6: High magnification view of fibrogenesis at the end of wrapping cytoplasmic process of Schwann cell. Numerous fibers directly contact with the end of cytoplasm, of which portion is devoid of distinct cell membrane or basement membrane. Number of fibrils and microtubular structures are contained in the cytoplasm. There are two slender fibroblastic cytoplasms in the periphery of figure.
Fig. 7: Tumor is entirely composed of spindle shaped or elongated cells and devoid of palisades. Note a characteristic feature that a tumor cell is enfolding another cell, as if it were a cut surface of capillary (arrows). There are numerous delicate connective tissue (reticulin) fibers in the intercellular space.