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Ultrastructural Aspects of Myogenesis Found in Neoplasms

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Summary. The ultrastructure of myogenic cells occurring in neoplasms was investigated and aspects of differentiation (myofilament interactions and organization, and sarcotubular development) were characterized. The stages of muscle differentiation present were extremely similar to those reported during human fetal development prior to innervation. Exceptions, however, being: (1) the presence of fewer multinucleated cells; (2) the general lack of cell elongation and its apparent effect on myofibril orientation; and (3) evidence of a higher number of myofilaments in mononucleated cells. The findings were compared to those reported in normal human fetal development, human myogenic cells in vitro and the literature on mammalian and avian muscle development and discussed with regard to the influence of tension and innervation. The significance of degenerating myogenic cells found in these neoplasms is also discussed.

Key words: Myogenesis – Neoplasms – Ultrastructure

Introduction

Developing skeletal muscle cells are found in a number of different neoplasms. Although the presence of these cells has been well documented (e.g., in [3, 20, 24, 33, 39, 43]) and in a few studies [16, 19, 25, 32, 36, 42], as well as in experimental systems [9, 12, 29], comparisons drawn between them and normal myogenesis, no critical evaluation of the differentiation present in these abnormal conditions with normal human myogenesis has been reported. In this study the development of muscle characteristics, sarcotubular as well as myofibrillar, in neoplastic cells was compared with the progression of events seen in normal myogenesis, as reported in human fetuses [35] and with the literature on "lower" animals [1, 2, 6–8, 11, 13, 17, 21, 28, 31, 34, 37].

Methods

Four tumors which contained myogenic elements were recently obtained at Children's Hospital (Columbus, OH, USA) and examined by electron microscopy: A cerebellar tumor (myomedulloblastoma); a renal tumor (nephroblastoma) and two rhabdomyosarcomas of the neck. The specimens were fixed in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide. Thin sections were stained with uranyl acetate and lead citrate and viewed with a Hitachi HS-7 (50 kv) electron microscope.

Results and Discussion

Myofibrillogenesis

The cellular levels of differentiation observed in myogenic cells from these neoplasms were strikingly similar to the normal progression of events during myogenesis in human fetuses [35], in explanted human tissue in vitro [22] and in the well studied chick embryo [1, 2, 8, 28]. The following categories of cells, based on the degree of organization of the myofibrils, suggest a developmental sequence:

1) Initial Muscle Characteristics. Relatively primitive cells were found which contained an electron-lucent cytoplasm, scattered profiles of smooth endoplasmic reticulum, thin filaments, and a paucity of thick filaments (occasionally none in the plane of section). The literature on normal myogenesis is not consistent with regard to whether thin filaments appear first [1, 28] or whether thick and thin appear concurrently [8]. These cells also contained filaments which appeared intermediate in size (Fig. 1), similar to previous developmental reports [15, 35]. Patches of Z-line material were also present and were always in association with thin filaments.

2) Initial Filament Association. In the cells which contained apprecable numbers of thick filaments the thick filaments were found in association with thin

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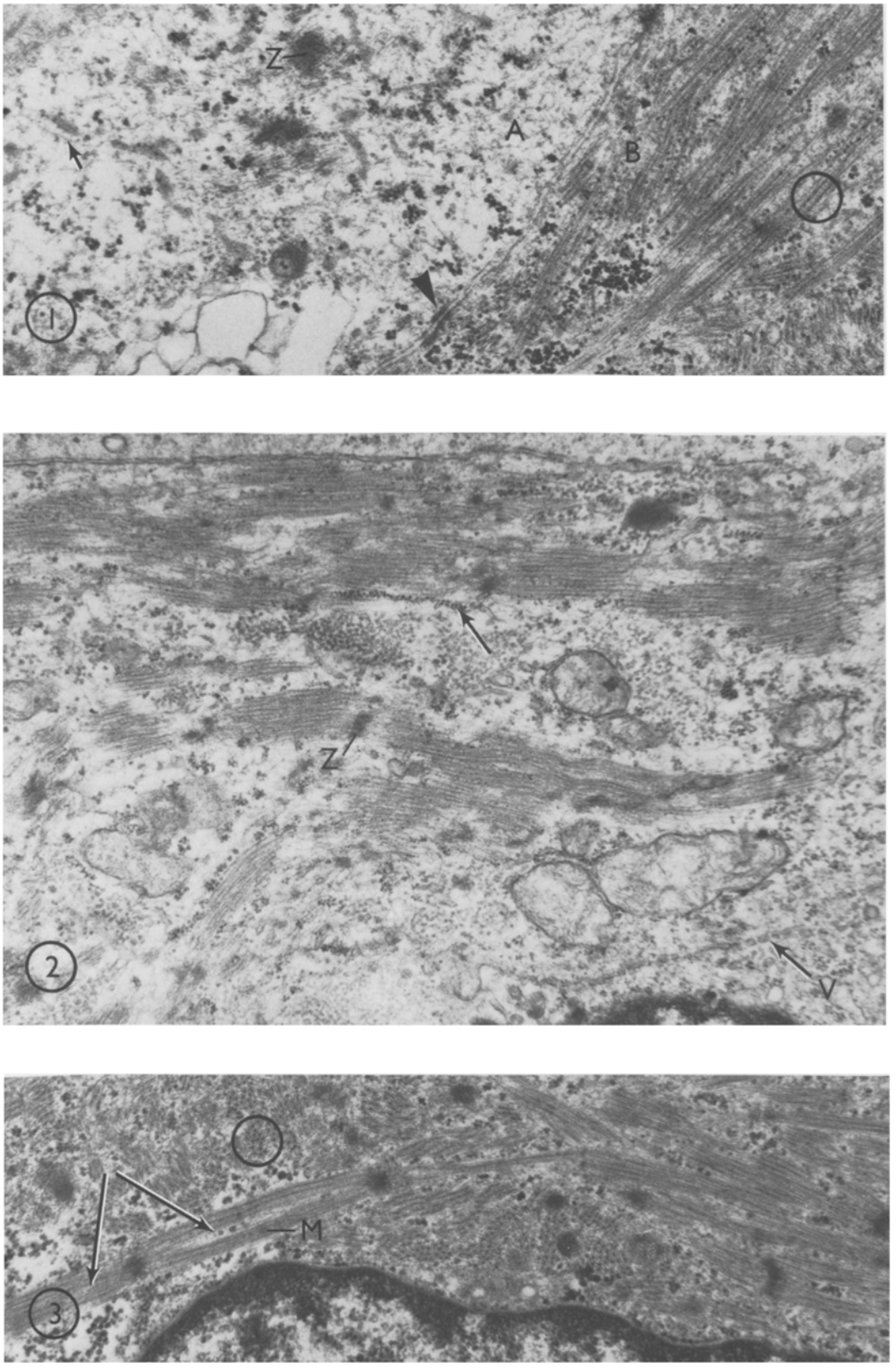


Figs. 1–3

filaments (Figs. 1, 2). This is consistent with normal myogenesis. The Z-line was not found to be necessary in thick and thin filament association. However, thin filaments were always in association with developing Z-lines. Often the cells in this stage of development contained long polyribosomes in association with the filaments (Fig. 2). Z-line appearance at this stage was in general blotchy.

3) "Early" Myofibril Organization. Cells in this stage were characterized by containing many filaments and exhibiting more organization, including primitive sarcomeres. At this level of organization M-lines were apparent. Also, the sarcomeres were found to be alligned in series more frequently than in parallel (Fig. 3). Z-line morphology varied from blotchy to relatively well developed.

4) "Late" Myofibril Organization. Cells were found which contained many filaments and, at least in regions, relatively good organization (Figs. 4, 5). On occasion, cells exhibited excellent parallel organization of well formed sarcomeres with M-line periodicity (24.5 nm) and Z-line morphology fully developed (Fig. 6). Tomanek and Colling-Saltin [35] found the presence of well formed Z-lines in register to be a late event during normal fetal development (approximately 30 weeks). The organization of elements of sarcoplasmic reticulum (SR), mitochondria, and glycogen around the fibrils was also notable (Figs. 5, 6).

The differentiation of muscle characteristics did not appear related to cell fusion. Myofibrillar development was observed in single cells as well as in fused cells with multiple nuclei. Although it is known that in vivo most synthesis of contractile proteins occurs after fusion [5] it has been shown that fusion is not a prerequisite for production of contractile proteins [14, 37]. The degree of asynchrony in cell differentiation found in these neoplasms is consistent with myogenesis in vivo.

The blotchy appearance of the Z-line in these poorly differentiated cells is consistent with reports on the early stages of fibril development in human [35] and chick [2] studies. In these neoplastic cells the fully developed Z-line architecture of filamentous and matrix components was not uniform throughout the cell until a relatively late stage in myofibril development. The establishment of a specific width of the Z-line is the final step in the differentiation of Z-line morphology as seen in the adult muscle fiber [34]. This, however, is a late development and appears dependent to a large degree on innervation [10, 34].

The orientation of the developing fibrils appeared greatly dependent on cell shape, with elongated cells exhibiting well ordered sarcomeres and the irregular or "rounded" cells exhibiting generally little organization. When organization was present in the rounded cells the fibrils were oriented in a concentric manner. Carlson [4] has shown that tension is necessary for cell elongation and orientation during regeneration of minced muscle. Although the initial elongation of a myoblast is a function of microtubules [40], the lack of tension is likely of major influence in the general lack of cell elongation and subsequent myofibril alignment in these tumors.

Sarcotubular System

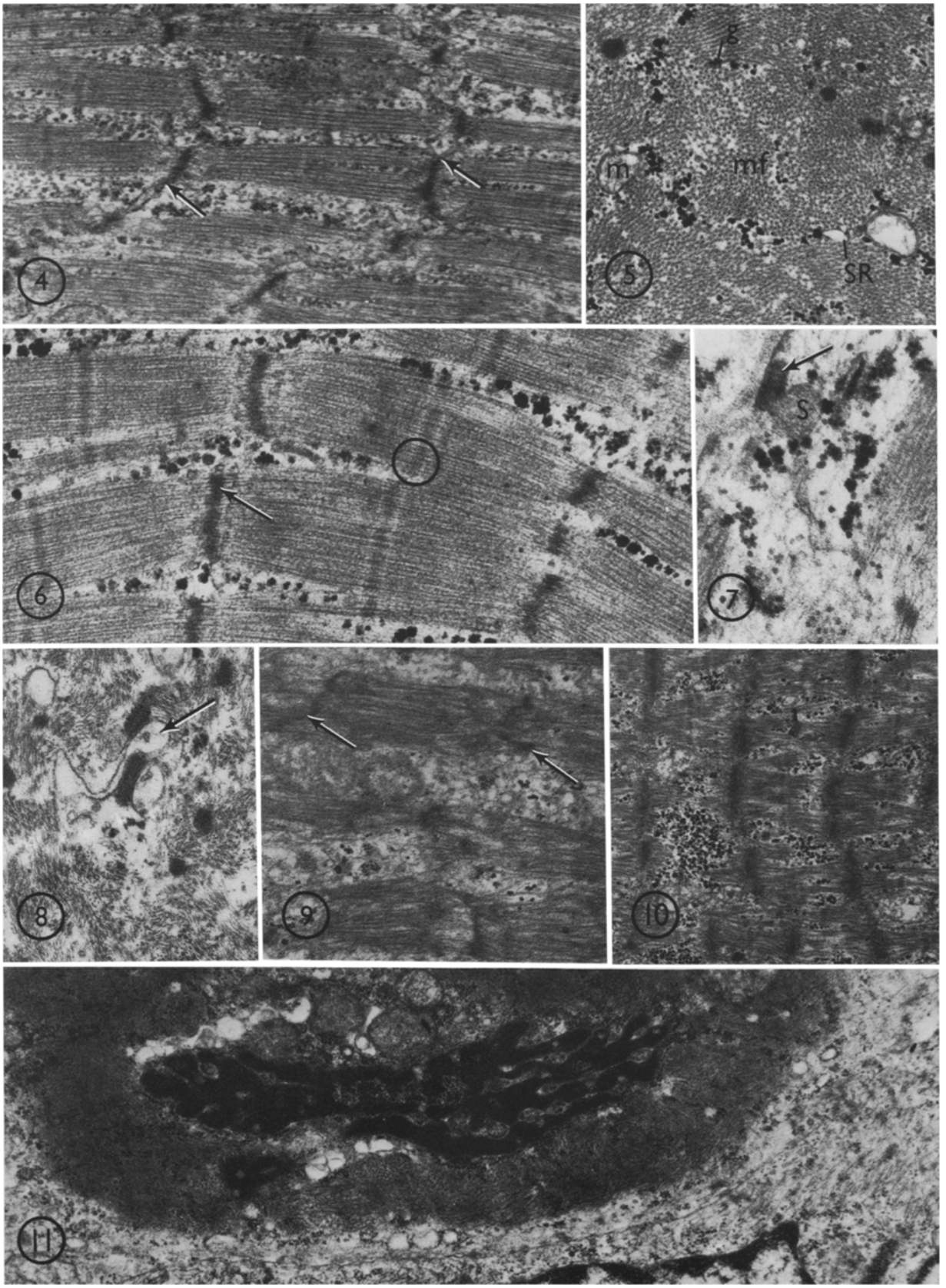
In contrast to the degree of sarcomere development, the sarcoplasmic reticulum (SR) and transverse tubule system (TTS) were rather poorly differentiated. This is similar, however, to the normal situation during human fetal development [35]. The most primitive configuration of the SR was the appearance of scattered profiles of smooth surfaced endoplasmic reticulum which appeared to form an extensive framework throughout the electron-lucent cytoplasm (Fig. 1). In general, as the myofibrils developed, the SR remained relatively undifferentiated in morphology, but did appear to ensheath the developing fibrils. Evidence of a fenestrated collar region was rare. The rather frequent finding of an association of the SR with the Z-line (Fig. 4) has also been reported in developmental studies [35, 38] and is of unknown significance.

Structures resembling terminal cisternae (TC), with an electron-dense substructure, were observed, being numerous in one neoplasm, while rare in the other

Fig. 1. Two neoplastic cells, connected by zonulae adherens (*large arrow*), are indicated. Cell "*A*" demonstrates initial muscle characteristics, containing thin filaments, patches of Z-line material (*Z*), and filaments intermediate in size between thick and thin filaments (*small arrow*). Cell "*B*" contains thick filaments which, even in this rather undeveloped cell, are alligned in parallel with thin filaments (e.g., see *circled area*). ×21,200

Fig. 2. Initial filament association. Note the long polyribosomes (*arrow*) in association with the filaments and the sporadic presence of Z-line material (*Z*). The presence of vesicles (*V*), in place of apposed membranes, is evidence of cell fusion. ×17,700

Fig. 3. Early myofibril organization. The developing sarcomeres are narrow and found in series (*arrows*). M-line formation is also evident (*M*) at this stage. The association of thick and thin filaments is seen in longitudinal and cross (*circle*) section. ×19,500



Figs. 4–11

three. With few exceptions these TC were found in association with the sarcolemma, forming diads (Fig. 7). The association of SR with the sarcolemma during early development has been noted by Tomanek and Colling-Saltin [35] in human fetal development. In the more developed cells the TC were infrequently found in association with what appeared to be elements of the TTS (Fig. 8). The TTS has been shown to originate via invaginations of the sarcolemma [7]. Using human material Tomanek and Colling-Saltin [35] have shown limited TTS development in vivo prior to innervation. The neoplastic myogenic cells in the present study also had minimal evidence of such invaginations.

Abnormalities and Degeneration

Infrequent cells exhibited anastamosing, irregular appearing myofibrils (Fig. 9) in which the normal, ordered appearance of the thick-thin filament assembly was missing. The Z-lines were non-descript in the filament masses and resembled the Z-line streaming found infrequently in healthy individuals [27] and commonly in numerous neuromuscular disorders [26].

Cells with supercontrated sarcomeres (Fig. 10) and degenerating, electron-dense cells containing masses of filaments (Fig. 11) were present to varying extent in these neoplasms. It is possible the degenerating cells are a result of inadequate blood supply, as is the cause of cell necrosis in many tumors. Often, however, the degenerating cells are found adjacent to normal cells. It is of interest that both supercontracted and degenerating cells are found during normal muscle morphogenesis [11, 18, 41], and furthermore that selective cell death is an important mechanism in morphogenesis [30]. This raises the possibility of unknown environmental cues being of effect in this degenerative phenomenon.

Degree of Differentiation — Practical Application

The finding of a qualitatively similar differentiation of the contractile constituents in these neoplastic myogenic cells with those of normal myogenesis suggests that quantitative differences between tumors may be more useful in any critical attempt to categorize rhabdomyosarcomas at the ultrastructural level. Such quantitative differences in myofibril development may be found at the tissue (e.g., number of cells exhibiting advanced fibrils) or cellular (e.g., increased Z-line material) level. Qualitative differences in the sarcotubular system may be useful. The lack of a well developed sarcotubular system is also useful in distinguishing the more highly developed, neoplastic muscle cells from normal or degenerating muscle fibers.

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Fig. 4. Late myofibril organization. Myofibrils are arranged in parallel with Z-lines relatively well developed and in register. An association of the SR with the Z-lines is present (*arrows*). ×15,200

Fig. 5. Late myofibril organization (cross-section). Glycogen (*g*), mitochondria (*m*) and elements of the SR are found surrounding the myofibril (*mf*). ×20,000

Fig. 6. Late myofibril organization. Excellent organization of sarcomeres, exhibiting well formed Z-lines (*arrow*) and M-line periodicity (*circle*). ×28,600

Fig. 7. A cisternae of SR (*arrow*), similar in structure to the terminal cisternae, with an electron dense substructure, is seen attached to the sarcolemma. Periodic densities are present, connecting the sarcolemma with these terminal cisternae. A continuity with the general network of SR (*S*) is evident. ×34,200

Fig. 8. Terminal cisternae in association with what appears to be elements of the transverse tubule system (*arrow*). ×23,200

Fig. 9. Cell exhibiting irregular bands of filaments with prominent Z-line "streaming" (*arrows*). ×15,800

Fig. 10. A myofiber exhibiting "supercontracted" (thick filaments pushed into the Z-line) myofibrils. ×10,900

Fig. 11. A degenerating cell exhibiting an electron-dense cytoplasm, masses of filaments, an irregular dense nucleus, and swollen vesicles in the perinuclear region. ×15,300

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