Nickel-Induced Malignant Tumors

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ABSTRACT

A series of malignant tumors experimentally induced by implanting Ni$_3$S$_2$ in gelatin capsules into the right hind limb of Fischer 344 rats is described. In this series, histology and ultrastructure revealed two major cell types. These are described as fibroblast-like and histiocyte-like. In addition, numerous giant cells, including large bizarre multinucleate giant cells with granular cytoplasm, were also seen. In this preliminary report, no more precise diagnosis will be given for this tumor than "malignant tumor of pluripotential origin". Further work is proposed to define more specifically its histogenesis. The appearances of the experimentally produced tumor are compared with certain tumors found in humans and also in experimentally induced tumors by Maruyama et al designated as malignant fibrous histiocytoma (MFH).

Introduction

The carcinogenicity of nickel and a number of its compounds has been the subject of many studies. In experimental animals, nickel carbonyl is carcinogenic when inhaled. Nickel subsulphide, which is very insoluble, appears to have the greatest carcinogenic potential when implanted in striated muscles or the testes of experimental animals. Rhabdomyomas and rhabdomyosarcomas have been the tumors most frequently reported following muscle implantation. Gilman has suggested a tissue preference by Ni$_3$S$_2$ for striated muscle, but other workers have not confirmed this. The present studies are confined to the tumorigenicity of Ni$_3$S$_2$ implanted into striated muscles of rats.

Materials and Methods

Seventy-eight tumors arising in Fischer 344 rats used in three separate experiments were available for study. The results of experiments 1 and 2 have already been published. No significant structural differences could be determined that were related to sex, treatment or castration. The results of the morphologic observations will, therefore, be reported together. In addition to the 78 primary tumors, metastatic deposits from 13 animals were examined. One tumor was transplanted successfully and
examined. This tumor failed to respond to a second generation transplant.\textsuperscript{21}

Ten mg of Ni\textsubscript{3}S\textsubscript{2} were loaded in 0.0 gelatin capsules. Following CO\textsubscript{2} anesthesia, small skin and muscle fascia incisions were made and the gelatin capsules were inserted into the body of femoral muscle in the right hind limbs of rats. Ten control animals were implanted with empty gelatin capsules. No tumors occurred in the control group.

Tumors were examined at death or sacrifice from all animals and those areas which had not undergone necrosis were used for more detailed analysis from 15 animals. Tumor material was fixed in buffered formalin (pH 7.0) and embedded in paraffin wax and methacrylate for routine and ultra thin (one micron) sections.\textsuperscript{13} Material fixed first in 2.5 percent glutaraldehyde and then in 1 percent osmium tetroxide was embedded in Spurr for electron microscopy. Special stains, including periodic acid-Schiff (PAS) with and without diastase digestion, alcian blue, Masson trichrome, toluidine blue and silver impregnation for reticulin, were employed as required.

**Results**

**Light Microscopy**

Spindle shaped cells were seen in varying proportions in all tumors, and these frequently were arranged in the so called storiform (cartwheel) pattern. Sometimes at the center of such a formation, a small vessel was found. Most of the cells arranged in the storiform manner formed collagen which was positive with Masson's stain. In some areas they were associated with myxoid material, which stained with alcian blue. These cells were identified as fibroblast-type (figure 1).

A variety of ovaloid and atypical giant cells were seen. Some very large bizarre giant cells occurred. All of these cell types had granular cytoplasm, best seen in ultra-thin sections stained with toluidine blue. These cells were considered to be of histiocyte-type (figure 2). Mitoses were frequent in all cell types. In tumors which had undergone softening,—a relatively frequent occurrence after six to eight months progression,—necrosis was common.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{A mixture of spindle cells and ovoid cells in a tumor produced by nickel sub-sulphide which developed five months after muscle inoculation. The storiform pattern of the spindle cells is seen here. H & E \times 100.}
\end{figure}
Infiltration of surrounding normal muscle with penetration between bands of fibers was almost always found. In these areas, degenerating muscle fibers assumed bizarre appearances. Occasional degenerate muscle fibers were found in more central parts of the tumors, but these could never be identified as part of the proliferating tumor mass. Careful search for Z bands of striated muscle type in undoubted tumor cells was negative in all cases except one. In this case, one or two cells with cross striations were seen which did appear to be in tumor cells (figure 3).

In the metastases and in the transplanted tumor, undifferentiated cells were found which were of short spindle or ovaloid shape. Occasional storiform arrangements were observed even in metastases.

Scattered through the tumors, and best seen in the ultra thin sections stained by toluidine blue, was red staining coarse granular material, sometimes in the cytoplasm of histiocytes. This is presumed to be particulate nickel. Further investigation is required to prove this point.

Although the two cell types (fibroblast-like and histiocyte-like) are described separately, nevertheless, they were found diffusely mixed throughout the tumors in many areas and in some areas groups of individual cells were found. A number of sections from tumors were stained with PAS both with and without diastase digestion. In all cases these were negative.

**Electron Microscopy**

Tumor material from six animals was examined. A minimum of three blocks was taken from each tumor. The cells designated fibroblast-like were spindle shaped and contained very well defined rough endoplasmic reticulum (RER) with prominent ribosomes. Dilated cisternae were frequent and many contained dark
amorphous material. Abundant formed and immature collagen was seen in the extracellular matrix in direct relationship to these cells. The nuclei were elongated and the nucleoli prominent (figure 4). Microfilaments with associated small focal dense bodies were seen occasionally. These were of the type seen in cells described as myo-fibroblasts\textsuperscript{12} (figure 5).

The histiocyte-like cells had many shapes but were never truly spindle shaped. Cell surfaces were irregular and microvilli frequent. The RER was not so well developed as in the fibroblast-like cells, but ribosomes were prominent. Golgi complexes were identified and well developed as opposed to the fibroblast-like cells where they were difficult to find. A striking feature of the histiocyte-like cells was the presence of dense bodies in the cytoplasm. Some of these were clearly lysosomes, but others showed no evidence of being membrane bound (figure 6). The presence of mitoses and multinucleated giant cells was confirmed by electron microscopy. Mitochondria were seen in both cell types but were not abundant and had no remarkable features.

Intermediate cells were seen between the two most common types already described. These were ovoid in shape but had extensive RER with large cisternae, well defined ribosomes and numerous intracytoplasmic dense bodies and lysosomes. Scattered cells showed

Figure 4. Electron micrograph of a fibroblast-like cell is shown with elongated nucleus, well defined nucleolus, abundant rough endoplasmic reticulum and extra-cellular collagen. Mag. × 11,850.
Figure 5. Electron micrograph of a fibroblast-like cell with microfilaments is shown with dense staining material lying across clumps of filaments. Extra-cellular collagen is well seen. These cells are designated as myo-fibroblasts rather than rhabdomyoblasts. Mag × 23,300.

crystalline precipitates of acid phosphatase containing material, in lysosomes and myelin figures. These occurred mostly in histiocyte-like cells. There were scattered undifferentiated cells with high nuclear-cytoplasmic ratio and few distinguishing nuclear or cytoplasmic details.

Discussion

The purpose of this preliminary report is to point out the difficulties of making a precise diagnosis of the tumor initiated by nickel subsulphide in the muscle of rats. It is difficult to exclude with confidence the possibility of a rhabdomyosarcoma, which has been the most popular diagnosis. Certain features of leiomyosarcomas are present, and sometimes this designation has been applied by other workers to these tumors. The difficulties of excluding degenerating muscle fibers and differentiating them from developing tumor cells has been discussed in relation to our series. Our conclusion is that the most likely event is the development of a tumor from pluripotential cells, so that a variety of cell types may be identified in the proliferating tumors. For these reasons it is proposed at the present time to describe the experimentally produced tumors simply as malignant tumors of pluripotential origin.

The histopathologic features found in these experimental tumors, the storiform pattern, the admixture of fibroblast-like and histiocyte-like cells, and the electron microscopic demonstration of myo-fibroblasts, are very similar to those seen in the human tumor known as malignant fibrous histiocytoma (MFH). It is also interesting that many of the findings in our cases are very similar to those described in a series of experimental subcutaneous tumors which were designated MFH. These were induced by local applications of 4-(hydroxyamino)-quinoline 1-oxide (4HQO) in Fischer 344 rats.10,14

It is recognized that making comparisons between experimentally induced tumors in animals and naturally occurring tumors in humans is fraught with difficulty. However, the problems described here in arriving at a specific diagnosis are reminiscent of those which have been associated with the definition of histogenesis in MFH.

Malignant fibrous histiocytoma in humans was first described in 1964 and 1967.15,17 It soon became apparent that many of the previously diagnosed poorly differentiated soft tissue sarcomas, including rhabdomyosarcomas, were more properly included in this category. Subsequent reviews11,25 have confirmed this and established MFH as one of the most frequently diagnosed soft tissue sarcomas occurring in man. The exact origin of the cells in these tumors is not fully understood at the present time. It was originally considered15,17 that all the cell types in the tumors arose from histiocytes and that some of them could take on the appearance of fibroblasts (facultative fibroblasts). As more electron microscopic studies have become avail-
able, some doubts have arisen as to the histiocytic origin\textsuperscript{4,24} and the possibility of origin from a pluripotential primitive mesenchymal cell has been considered.\textsuperscript{16} The myofibroblasts seen in our series have been reported in human MFH.\textsuperscript{2} Myofibroblasts have also been reported in fibrosarcomas.\textsuperscript{7} A concept has been suggested\textsuperscript{9} that a spectrum of human tumors exist, which can show a variety of specialized features varying from fibroblast-type to myogenic-type including histiocytic-type cells. This would explain the fact that fibroblastic RER has been reported in leiomyomas and leiomyosarcomas.\textsuperscript{3} That concept would be in agreement with a proposed histogenesis of MFH from mesenchymal cells showing differentiation into the three forms of histiocytic, fibroblastic, and myofibroblastic types.\textsuperscript{4}

Such a concept is attractive in considering the histogenesis of the experimentally produced tumors in our series and might serve as a basis for the reason that workers have described different tumor types that develop after muscle implantation of Ni\textsubscript{3}S\textsubscript{2}. It seems appropriate at the present time to regard these tumors, experimentally induced in muscle with Ni\textsubscript{3}S\textsubscript{2}, as malignant tumors arising from mesenchymal cells with pluripotential characteristics. Careful study to establish the possible presence of rhabdomyo-
blasts, leiomyoblasts, fibroblasts, and histiocytic cells must be performed using more sophisticated methodology. Such studies may make it possible to shed further light on the histogenesis of human MFH by using an animal model such as the one described here.

References


