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Identification of Proliferative Lesions in Mollusks

THOMAS C. CHENG

In recent years, there has been accelerated interest in the occurrence of proliferative and other types of neoplastic growth in mollusks. Consequently, although earlier summaries of reports of neoplasia in this group of invertebrates are available (Scharrer and Lochhead, 1950; Wautier, 1955; Wautier and Wautier, 1953; Pflugfelder, 1954), the number of reports has increased dramatically since the mid-1950's (see Pauley, 1969, for review). The question that needs to be asked is: Are all of the reports truly of neoplasia or are some histopathologists misinterpreting what they have observed? The reason for raising this question is because it is my opinion that some are misinterpreting leucocytic granulomas in tissue sections as proliferative neoplasms. This is an easy error to make since, morphologically, islands of immunologic cells could resemble atypical proliferating cells, even at the electron microscopical (EM) level. Specifically, at one time it was thought that finding at the EM level of elongate cytoplasmic extensions from the members of seemingly abnormal nests of cells was a reliable diagnostic feature of proliferative neoplastic cells. This idea, however, has since been discredited by Harris (1975) at this laboratory who found that certain cells contributing to the encapsulation process in the pulmonate gastropod *Biomphalaria glabrata* experimentally infected with the nematode *Angiostrongylus cantonensis* typically produce such filopodia-like projections.

Cheng

Thomas C. Cheng is with the Institute for Pathobiology, Center for Health Sciences, Lehigh University, Bethlehem, PA 18015. *The original research data for this paper resulted from studies supported by grants FD-00416 and AI-12355-01A1 from the U.S. Public Health Service.*

It is recalled that the internal defense mechanisms of mollusks are primarily cellular, i.e., intra- and extravascular phagocytic hemolymph cells, which, upon recognizing the invading material as nonself, either successfully or unsuccessfully phagocytose such material. It is generally believed that if the foreign substance is dimensionally too large to be phagocytosed, then it becomes encapsulated. Although it has been suggested by Cheng and Rifkin (1970) that encapsulation may represent unsuccessful attempts of phagocytosis by many cells, experimental proof of this remains to be established. One approach to resolving this problem is to find chemical markers which would permit the identification of phagocytosis in histological sections, and see if such specific markers also occur in cells involved in encapsulation.

It must also be recalled that encapsulation may lead to the destruction and resorption of the nonself material, and when this occurs, the nonself material is no longer discernable, but an islet of the cells that comprised the original capsule remains for sometime (Cheng and Rifkin, 1968). These cells, in my opinion, comprise the type of histopathological picture that has been commonly misinterpreted as proliferative neoplastic cells. To distinguish between these reaction cells and true neoplastic cells, again, chemical markers could be useful. At the Institute for Pathobiology we have been searching for such markers, and the following is a brief review of our findings to date.

PHAGOCYTIC CELLS

Cheng and Rodrick (1975) have detected and quantified the activity levels of β-glucuronidase, acid phosphatase, alkaline phosphatase, lipase, and lysozyme, all lysosomal enzymes, in resting phagocytes of the pelecypods *Mercenaria mercenaria* and *Crassostrea virginica.* It was deemed of interest to determine whether these enzymes could be employed as markers to distinguish between phagocytes and neoplastic cells. It has since been found that lysozyme is a good marker for active phagocytes of *C. virginica* (Rodrick and Cheng, 1974), *Mya arenaria* (Cheng and Rodrick, 1974), and *M. mercenaria* (Cheng et al., 1975). Furthermore, it has also been determined that there is elevated lipase activity in active phagocytes of *M. arenaria* and the gastropod *B. glabrata* (Cheng and Yoshino, 1976 and in press), and there is a similar elevation of aminopeptidase activity in actively phagocytosing cells of *C. virginica.* It remains unknown, however, whether proliferative neoplastic cells in these species of mollusks include detectable levels of these lysosomal enzymes. If not, then cytochemical and/or biochemical identification of these hydrolases could serve to facilitate differentiation between phagocytes and pathologic cells.

ENCAPSULATION CELLS

Rifkin and Cheng (1968) have found that the encapsulation complex formed in *C. virginica* in response to parasitization by *Tylocephalum* metacestodes include glycoproteins and/or mucoproteins and neutral polysaccharides. Also, Harris and Cheng (1975) have revealed that the cellular encapsulation complex in *B. glabrata* parasitized by *Angiostrongylus cantonensis* is rich in acid phosphatase and nonspecific esterase as well as lesser amounts of alkaline phosphatase and β-glucuronidase.

Cheng (1975) has reported that the encapsulation complex in *Crassostrea gigas* formed in response to the nematode *Echinocephalus crassostreai* includes complex carbohydrates that are sulfated and rich in acidic groups, glycogen, carboxylate polyanions, and one or more of the following: neutral mucopolysaccharides, mucoproteins, and glycoproteins.

Again it is not known whether proliferative neoplastic cells include the molecules associated with cells and/or fibers involved in encapsulation in mollusks. If such do not occur, these molecules could serve as markers to distinguish between these types of cells.

RECOMMENDATION

It is being recommended strongly that the identification of neoplasms in mollusks not be based solely on gross and conventional histopathological observations since such could lead to misidentification. The use of molecular markers could serve as a useful tool in distinguishing cells involved in immunologic response and true neoplasms.

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