Establishment of a Cell Line from the Platyfish-Swordtail Hybrid Melanoma

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ABSTRACT

A fish melanoma cell line, PSM-1, was established from a hereditary melanoma in an interspecific hybrid of Xiphophorus for cytogenetic studies of this melanoma system. An amelanotic melanoma was obtained from the tail fin of a melanotic F1 hybrid between a spotted platyfish (Xiphophorus maculatus) and an albino swordtail (Xiphophorus helleri). The tumor tissue was dissociated and cultured in Eagle’s minimal essential medium supplemented with 10% fetal calf serum. PSM-1 has been maintained for 52 passages and 29 months in vitro since March 3, 1978. This cell line showed considerable variation in cellular morphology. Although no apparent pigment was detectable by light microscopy, melanosomes and premelanosomes could be seen under the electron microscope. The cells grew randomly across each other with an apparent lack of contact inhibition and formed many clumps. The doubling time was approximately 2 days, and the modal chromosome number at the 41st passage was a near triploid 71. A high tyrosinase activity was seen under the electron microscope. The cells grew randomly from an inoculum of 105 cells/35-mm plate during 11 days. Cell counts were made on triplicate plates with a hemacytometer.

INTRODUCTION

Malignant melanomas occur spontaneously with high frequency in interspecific hybrids between the platyfish carrying genes for macromelanophores (a large type of melanophore) and the swordtail. The hybrid melanoma has been used as a model for investigating the role of genetic factors in cancer etiology (1, 4). It has been hypothesized that alterations in gene combination in germ cells through hybridization predispose the hybrid fish to melanomas (1). Although this hypothesis is based mainly on genetics at the individual level, cytogenetic studies provide more precise information on genetic mechanisms of the melanoma system. Some attempts have been made to culture the melanomas in vitro (5, 6, 9). No such cell line, however, has been established yet. The present paper reports the establishment of a cell line from the hybrid melanoma; this is the first cell line of fish melanomas.

MATERIALS AND METHODS

Cell Culture. An amelanotic melanoma was obtained from the tail fin of an adult melanotic F1 hybrid between a spotted platyfish (Xiphophorus maculatus) carrying a macromelano-

RESULTS AND DISCUSSION

In the early stage of the culture, bipolar and polydendritic cells were spread out homogeneously and overlapped each...
other. Mitotic figures could often be observed. Subcultures were made at 1-week intervals without splitting. One month after the primary culture, however, most of the cells formed clumps which tended to float away. No mitotic figure could be observed during this period. After 4 months, cellophane membranes were placed over the clumps; consequently, the clumps became attached to the plates. The cultures, however, were quiescent under the cellophane membranes for an additional 6 months. After 10 months, the cells emigrated from the clumps and proliferated, forming chains consisting of many attached cells. The cellophane membranes were removed after 21 passages and 18 months, since the cells could attach to the plates and proliferate actively without these membranes. During recent months, subcultures were made at 1-week intervals at a 1:3 split ratio. The established cell line was designated PSM-1 and has been maintained for 52 passages and 29 months in vitro since preparation of the primary culture on March 3, 1978.

PSM-1 showed considerable variation in cellular morphology. Many cells were bipolar or polydendritic with the major axis of 70 to 200 \( \mu \text{m} \) or round with a diameter of 20 to 60 \( \mu \text{m} \). Some were stellate (50 to 100 \( \mu \text{m} \)) or epithelial-like (50 to 300 \( \mu \text{m} \)). The cytoplasm appeared dark and often granular with no apparent melanosomes under phase-contrast microscopy. Electron microscopic observations revealed melanosomes and premelanosomes which closely resembled those in differentiating fish melanophores (11). Round or ovoid nuclei were from 7 to 20 \( \mu \text{m} \) in diameter with one or more nucleoli. Multinucleated cells were sometimes seen. Giant lobulated nuclei were often observed in large epithelial-like cells. The cells grew across each other at random with an apparent lack of contact inhibition. Many clumps of the cells were formed in actively proliferating cultures and often became detached from the plates. The cells were easily separated from the plates by gentle pipetting. The culture medium became acidic to a considerable extent. The population-doubling time was approximately 2 days. The chromosome number at the 41st passage ranged from 53 to 167 with a mode of 71 which was near triploid (3). Many cell lines have been established in mammalian melanomas (2, 8). PSM-1 is similar to some of these cell lines with respect to cytological characteristics and growth patterns. This cell line provides a useful means for carrying out cytogenetic studies on the hybrid melanoma and for making comparative studies on vertebrate melanomas at the cellular level.

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REFERENCES


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\(^{a}\) Mean value of 3 experiments.
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