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Research Note

Transformation of BALB 3T3 Cells by Schistosome Eggs

YUJI IRIE[1], KEIJI FUJII[2] AND YUKIO IWAMURA[3]

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It is generally accepted that schistosomiasis may be one of the etiologic factors inducing human hepatocellular carcinoma, urinary bladder cancer, lymphoma, leukemia and colon cancer, respectively (Cheever, 1978). However, direct causative agent to human cancer associated with schistosomiasis has not been identified yet.

In the animal model, Amano and Oshima (1988) reported that 48 out of 61 female ddY mice with chronic schistosomiasis japonica yielded hepatocellular carcinomas without the feeding of chemical carcinogen. Under similar conditions used in their experiment, we also observed that 3 out of 13 male ICR mice infected with *Schistosoma japonicum* and survived for maximum of 50 weeks developed hepatocellular neoplasm. On the other hand, it has been reported that enhanced expression of *ras* p21 proteins was detected by western blotting analysis in human bladder cancers with schistosomiasis haematobia (Fujita *et al.*, 1987). Therefore it seemed necessary to verify whether *ras* oncogenes are involved in tumorigenicity associated with schistosomiasis.

Since the main causative agent of the disease is due to the eggs accumulated in the liver and other tissues (Warren, 1982), we examined the transforming potential of schistosome eggs using BALB 3T3 cells (Aaronson and Todaro, 1968; Jainchill *et al.*, 1969; Aaronson and Weaver, 1971; Risser and Pollack, 1974). Eggs of *S. japonicum* and *S. mansoni* were collected by sieving method (Dresden and Payne, 1981) from the liver and intestine of male ICR mice 8–10 weeks after infection, followed by cultivation in RPMI 1640 containing 10% fetal calf serum for 3 days ($1–3\times10^4$ eggs/well/2 ml medium). The supernatant of egg culture medium of both species was obtained by centrifugation after freezing and thawing. The monolayer of BALB 3T3 cells (Fig. 1A) was exposed to the supernatant of egg culture medium of both species for 24 hr ($1–2\times10^6$ cells/well/2 ml supernatant). After washing by RPMI 1640, the cells were transferred to each petri dish (6 cm in diameter) and maintained for 21 to 28 days in RPMI 1640 containing 2% fetal calf serum for detection of the transformed colonies.

Exposure to the supernatant of egg culture medium of both species apparently induced morphological transformation of BALB 3T3 cells (Fig. 1B). The foci of transformed BALB 3T3 cells by the supernatant of egg culture medium of both species were isolated under an inverted microscope and propagated in RPMI 1640 containing 10% fetal calf serum. Then the transformed BALB 3T3 cells (5×10^6/mouse) were inoculated subcutaneously into BALB/c nude mice. Two weeks after inoculation, a small subcutaneous tumor nodule appeared in one of 3 mice inoculated with the transformed BALB 3T3 cells by the supernatant of egg culture medium of *S. japonicum*. The tumor enlarged in a size of 3×2 cm by 6 weeks after inoculation (Fig. 1C), showing a feature of hyperplastic nodule. However, no tumor was found in 3 mice inoculated with the transformed

Departments of [1]Parasitology, [2]Pathology and [3]Microbiology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba-shi, Ibaraki-ken 305, Japan.
入江勇治（筑波大学基礎医学系寄生虫学）
藤井敬二（筑波大学基礎医学系病理学）
岩村幸雄（筑波大学基礎医学系微生物学）

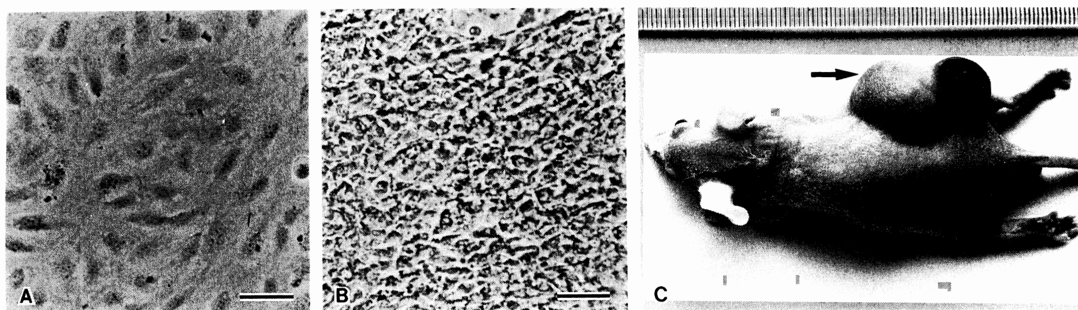

Fig. 1 Transformation of BALB 3T3 cells by exposure to the supernatant of schistosome egg culture medium.
A. The monolayer of control BALB 3T3 cells. B. The focus of transformed BALB 3T3 cells by the supernatant of *S. japonicum* egg culture medium. C. A tumor developed in the subcutaneous tissue of a nude mouse 6 weeks after inoculation of the transformed BALB 3T3 cells by the supernatant of *S. japonicum* egg culture medium.
Scale bars, 50 μm; Arrow, tumor.

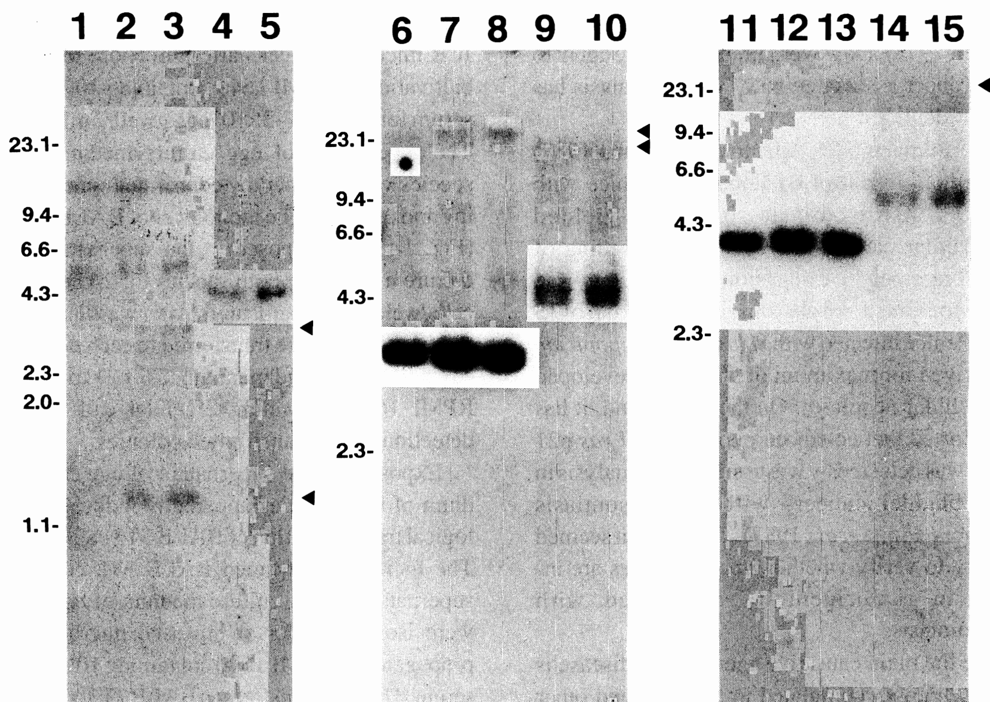

Fig. 2 Detection of Ha-*ras* oncogene in the DNAs of transformed BALB 3T3 cells and schistosome eggs.
DNAs were isolated by the method of Maniatis *et al.* (1982) and digested with *Eco*R I (Lanes 1–5) or *Bam*H I (Lanes 6–15). The ^{32}P-labelled probe used was a v-Ha-*ras* 0.46 kbp insert DNA (BS-9) (Ellis *et al.*, 1980). After hybridization, the filters were washed at 65°C (Lanes 1–5) in 0.2×0.15 M NaCl/0.015 M sodium citrate (SSC), 60°C (Lanes 6–10) in 0.2×SSC and 68°C (Lanes 11–15) in 0.1×SSC, respectively. Source of DNA: Lanes 1, 6, 11, control BALB 3T3 cells; Lanes 2, 7, 12, the transformed BALB 3T3 cells by the supernatant of *S. mansoni* egg culture medium; Lanes 3, 8, 13, the transformed BALB 3T3 cells by the supernatant of *S. japonicum* egg culture medium; Lanes 4, 9, 14, eggs of *S. mansoni*; Lanes 5, 10, 15, eggs of *S. japonicum*.
Closed triangles indicate additional signals.

cells by the supernatant of *S. mansoni* egg culture medium.

In oncogenes so far as examined by blot hybridization analysis (Southern, 1975), additional bands of Ha-*ras* oncogene were detected in the DNAs of the transformed BALB 3T3 cells by the supernatant of egg culture medium of *S. japonicum* and *S. mansoni* (Fig. 2, lanes, 2, 3; lanes, 7, 8; lanes, 12, 13). In contrast, these bands could not be found in the DNAs of the schistosome eggs (Fig. 2, lanes, 4, 5, 9, 10, 14 and 15). We further attempted to confirm involvement of Ha-*ras* oncogene in the process of transformation using NIH 3T3 cells (Jainchill *et al.*, 1969: JCRB, #0615), which is known as a sensitive cell line to identify activated *ras* oncogenes (McCoy *et al.*, 1983). However, no transformed focus was induced by exposure to the supernatant of culture medium of *S. japonicum* and *S. mansoni* eggs under the same condition used for BALB 3T3 cells. This result reveals that the transforming potential of schistosome eggs was not due to transfer of activated Ha-*ras* oncogene into BALB 3T3 cells.

Results obtained here indicate that Ha-*ras* oncogene in the transformed BALB 3T3 cells was activated by means of an alteration such as rearrangement caused by exposure to the supernatant of egg culture medium.

It has been recognized that *ras* oncogenes in human and animal cancers participate in the early stages of the multi-step of carcinogenesis. As shown here, activation of Ha-*ras* oncogene in schistosomiasis should provide definitive evidence regarding its genetic nature involving in hepatocellular carcinoma as well as urinary bladder cancer. Recently, Badawi *et al.* (1992) proposed a model for the induction of bladder cancer associated with schistosomiasis haematobia that alkylating agents such as the metabolites of N-nitroso compounds can contribute to the induction of this neoplasm. Although the factor derived from the component of schistosome eggs is still unknown, we presented here a fundamental clue for better understanding of tumorigenesis associated with schistosomiasis.

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