Growth conditions of F9 embryonal carcinoma cells affect the degree of DNA methylation

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Abstract

We have investigated differences in C*pG methylation between F9 embryonal carcinoma cells in vitro and as tumor cells grown in vivo using Msp I and Hpa II restriction isoschizomers. Southernns were hybridized with two low copy number probes, mouse major β-globin (β) and a class I, histocompatibility-2 cDNA clone (pH-2d-4). In each case, the tumor-DNA was hypomethylated while the DNA from F9 cells grown in vitro was moderately methylated. We conclude that growth conditions or cell-cell interactions can greatly affect methylation of C*pG sites.

Introduction

Many studies of site-specific methylation of structural genes have found hypomethylation of variably methylated sites in tissues or cells where the gene is expressed (16, 19, 21, 26, 27, 32) and hypermethylation in tissues where they are not expressed. Whether DNA methylation plays a role in the control of gene expression or if methylation occurs after regulation cannot be presently ascertained. In point of fact, many exceptions to this correlation have been found (9, 10, 18, 24, 35).

One of the exceptions to the degree of methylation of certain genes is that which occurs in transformed cells and tissues. Analysis of DNA from lung and colon tumors, with three specific probes to genes not expressed in these tumors, showed them to be hypomethylated when compared to their normal tissue counterparts (6). Because these tumors had not been adapted to tissue culture and were from untreated patients, hypomethylation of normally hypermethylated genes cannot be due to an experimental manipulation. In one case, several metastases were found to have increasingly hypomethylated patterns compared to the primary tumor. In contrast, a comparison of methylated C*pG sites in adult rat liver, a rat hepatoma line, and fetal rat liver using probes to albumin and alpha fetal protein genes showed no correlation in the changes in the methylation pattern with gene activation or repression (24). These data suggest that (1) the rapid growth of tissues affects the methylation pattern of various genes; (2) the relationship of cell-cell interactions in culture versus tumors of various tissues affect the degree of methylation; or (3) the morphology and state of differentiation of tumors change in comparison to their normal counterpart during tumorgenesis and metastasis.

We have investigated the first and second possibility, i.e., that growth and/or cell-cell interactions may influence methylation patterns in tissues and cells from tumors using embryonal carcinoma cells. In order to control for possible differences in differentiated and undifferentiated tissues, we used the F9 mouse embryonal carcinoma (EC) line. F9 is a ‘nullipotent’ EC cell line originally derived from a transplantable testicular tumor that arose in strain 129/J (1). Although considered ‘nullipotent’, treatment with trans-retinoic acid will stimulate almost all of the cells to differentiate to primitive
yolk sac cells (33). F9 EC cells grown subcutaneously in syngeneic hosts grow rapidly, forming well-defined tumor masses with only embryonal-like cells apparent.

We recently used F9 tumor DNA as a control during studies of DNA methylation during spermatogenesis (25) and found it to be hypomethylated when Southern's were probed with several cDNA clones, including to H-2. Unlike many other tumors, EC cells or tumors do not normally express class I histocompatibility antigens (2), H-2, unless differentiated (31) or transplanted to non-syngeneic hosts (23). In contrast, Morello et al. (22) had found a high degree of methylation of C*pG site at the H-2 locus in cultured F9 cells. In this study we compared the degree of methylation of F9 cells when grown in vitro and in vivo, using restriction isoschizomers. We present data herein that F9 nullipotent embryonal carcinoma cells are hypermethylated when grown in tissue culture, but hypomethylated when grown in vivo. Thus, growth conditions are likely to be an explanation for the variable methylation.

Materials and methods

F9 EC cellular DNA was obtained from cultures grown in Ann Arbor and Paris. The Ann Arbor cells, originally obtained from Dr. Peter Andrews, were grown in D-MEM (Gibco) containing antibiotics (penicillin and streptomycin) and 5% heat inactivated fetal calf serum. Cells were trypsinized and replated every three days to avoid accumulating spontaneously differentiating cells. The F9 and related PCC4/Aza cellular DNA from Paris was obtained as previously described (22). F9 tumors were induced by injecting approximately 1 x 10⁵ F9 cells (Ann Arbor) subcutaneously into 129/SvJ male mice. Tumors were taken when less than 1 cm in diameter; they were found to be typical of undifferentiated embryonal carcinomas in vivo. DNA was purified from both tumors and cells using the proteinase K/phenol method (11).

Restriction digests with Eco RI, Msp I, and Hpa II were performed as recommended by the manufacturer (Bethesda Research Laboratories and PL Biochemicals) using twice the recommended concentration for 24 hrs at 37 °C. The DNA was ethanol precipitated and resuspended in TE buffer and tracking dye. Electrophoresis was carried out in 1% agarose (BRL) at constant voltage (1.6 V/cm) in ‘E’ buffer as previously described (25). Transfer of DNA to nitrocellulose or Gene Screen® was performed as described by Southern (30). Hybridizations were performed at 42 °C in 5XSSC and 35% formamide.

Low copy number probes

The specific gene probes used were a mouse β-major globin clone, f7, [Dr. Carolyn Jahn, (14)], and a mouse class I, histocompatibility-2 clone, pH-2d-4, [Dr. Gabriel Gachelin, (15)]. Nick translations of the probes were done with the NEK-004C kit of New England Nuclear using [α32p]-dCTP at 400 mCi/mM. Any one figure represents strips hybridized with the same probe and exposed for the same length of time.

Results and discussion

Hybridization of Msp I and Hpa II restricted DNA from F9 tumor and cells with the mouse major β-globin probe showed the tumor DNA to be less methylated at C*pG sites (Fig. 1). The Hpa II pattern of F9 tumor DNA was nearly identical to that found after restriction with Msp I, differing by the presence of a 7.7 kb fragment with Hpa II not seen with Msp I. A previously prepared batch of F9 tumor DNA studied by these methods and with this probe had shown identical Hpa II and Msp I patterns (25). Thus, it is probable that there exists variability in the degree of C*pG methylation of DNA extracted from different F9 tumors similar to that existing between different F9 in vitro lines. F9 cell line DNA (Ann Arbor) showed Hpa II fragments of 13.5 and 7.7 kb and less of the 6.0 and 2.7 kb fragments seen in the Msp I digestion. The F9 cell line from Paris seemed even more methylated as only the 6 kb, and fainter higher M.W. bands, were seen with Hpa II digestion. All three DNA preparations showed similar Eco RI restriction patterns.

A similar result was found when the DNAs were probed with the class I, major histocompatibility complex cDNA clone, pH-2d-4 (Fig. 2). The F9 tumor showed nearly identical Msp I and Hpa II digestion patterns while F9, and PCC4 (a multipotent embryonal carcinoma cell line), showed varia-