EVALUATION OF IMMUNOHISTOCHEMICAL STAINING AND ACTIVITY OF THYMIDYLATE SYNTHASE IN CELL LINES

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INTRODUCTION

Levels of the enzyme thymidylate synthase (TS) in tumour tissue play an important role in the outcome of chemotherapy with drugs directed against TS. High TS levels may be predictive for resistance against these drugs¹. It has been shown, both *in vitro* and *in vivo* that high intrinsic TS levels correlate with resistance to 5-fluorouracil (5FU)^{2,3}. Moreover exposure of several neoplastic cells and tissues to 5FU cause a marked increase of TS levels^{4,5}. The common methods to measure TS in cells and tissues are enzyme assays on crude extracts⁶. The FdUMP binding assay determines the number of free FdUMP binding sites, while the tritium release assay, based on the conversion of [5-³H]-dUMP into dTMP and ³H₂O, provides data about the catalytic activity of TS. These assays require about 2.10⁷ cells. With a polyclonal antibody, raised against recombinant human TS (ahTS)⁷ the detection of TS protein levels in smaller numbers of cells is possible, either with ELISA or immunohistochemical (IHC) staining methods

METHODS

A panel of 13 cell lines (Table 1) was used for IHC staining and measurements of FdUMP binding and catalytic activity including 3 murine, 1 rat and 4 human colon cancer cell lines; 3 human cell lines derived from squamous cell carcinoma of the head and neck and 2 human lymphoblastoid cell lines, the parent W1-L2 and the resistant, TS overproducing W1-L2:C1⁸. We applied standard IHC staining methods.

Cytospins were fixed in 100% acetone. They were incubated with the first antibody, ahTS (1:300) for 1 h, and after washing, for 1 h with the second antibody (swine-anti-rabbit immunoglobulins conjugated to peroxidase, 1:100). The staining was completed by addition of 3,3'-diaminobenzidine (0.5 mg/ml) and H₂O₂ (3% solution). Two individuals scored independently the intensity of IHC staining of the cytopins from low (+) to high (++++).

Enzyme assays for FdUMP binding and catalytic activity of TS were performed as described by Peters *et al*⁶.

RESULTS

The lymphoblastoid cells had an intense staining at the rim of the cell, while the cytoplasm showed minimal staining (Fig. 1a-b). Two of the human colon cancer cell lines also stained stronger at the rim of the cell than in the cytoplasm, but all the other cells had a homogeneous staining of the cytoplasm. The ahTS also recognized rat and murine TS. The murine and rat cells showed a staining comparable to that of the human colon cancer cells.

Comparison of the intensity of the staining with the known catalytic activity or FdUMP binding of the cell lines revealed a good correlation of high intensity and high TS activity and high capacity of FdUMP binding to TS within the murine colon cancer cells and the human head and neck cancer cells (Table 1). For the head and neck cancer cell lines UM-SCC-14C with 379 pmol/h/mg protein (catalytic activity) and 158 fmol/mg protein (FdUMP binding) had a staining scored as (+), while those values for UM-SCC-11B were 2526, 864 and (++++), respectively (Fig. 1c-e). The staining of the resistant lymphoblastoid cells (+++) was stronger than that of the parent (+) (Fig 1a-b). Within the panel of the human colon cancer cell lines no clear relation could be detected. Interestingly two colon cancer cell lines (1 murine, 1 human), which have been adapted to low folate levels showed an increase in TS catalytic activity (1.5x and 2.5x, resp.) and intensity of IHC staining (1.25x and 2x, resp.) as compared to their parental line.

DISCUSSION

IHC staining of cytopins revealed two types of staining, cytoplasmic and cell rim. In most of the cell lines grouped according to origin a good relation between TS levels measured with classic enzyme assays and intensity of IHC staining was observed. A similar trend has been observed for results obtained by Western blotting with a monoclonal antibody against human TS and FdUMP binding⁹.

IHC staining for TS can be an additional method to indicate TS protein levels, but quantification of TS levels was disputable with this method. Quantification may be performed by densitometric measurements of Western blots or ELISA⁹. It has been shown for the parent and resistant lymphoblastoid cells that the difference in TS protein levels could also be detected by ELISA methods, with the polyclonal antibody⁷. The IHC detection of TS in tumor samples could be of prognostic value for patients which receive (adjuvant) FU therapy, since high TS levels seem to be correlated to resistance against FU.

Figure 1. Immunohistochemical staining with ahTS (1:300) of 2 lymphoblastoid cell lines W1-L2 (A) and W1-L2:C1 (B) and 3 human squamous cell carcinoma cell lines of the head and neck UM-SCC-14C (C) UM-SCC-22B (D) and UM-SCC-11B (E). Bar is 10 μ m.

Table 1
Comparison of intensity of IHC staining of TS
with FdUMP Binding to TS an TS Catalytic activity

	IHC cytopins	TS catalytic activity (pmol/h/mg protein)	FdUMP binding (fmol/mg protein)
<u>Human colon cell lines</u>			
SW620	++	358 ± 97	161 ± 17
WiDr	+++	675 ± 257	218 ± 98
HT29	+++	700 ± 281	206 ± 61
WiDr/F*	+++++	1556 ± 22	222 ± 93
<u>Rat colon cell line</u>			
CC531	++	1242 ± 87	712 ± 93
<u>Murine colon cell lines</u>			
C38-2	++	109 ± 27	289 ± 64
C26-10	+++	7100 ± 1300	3113 ± 512
C26-10/F*	+++++	9503 ± 1178	1756 ± 136
<u>Human squamous cell carcinoma cell lines of the head and neck</u>			
UM-SCC-14C	+	379 ± 177	158 ± 39
UM-SCC-22B	+++	969 ± 119	150 ± 19
UM-SCC-11B	+++++	2526 ± 1498	864 ± 339
<u>Human lymphoblastoid cell lines</u>			
W1-L2	+	5130 ± 203	152 ± 49
W1-L2:C1	+++	> 16206	5337 ± 24

* Cell lines derived from WiDr and C26-10, respectively, but adapted to grow in folate depleted medium (1 nM folinic acid). Values are means of 2 independent scores (IHC) or at least 3 enzyme assays

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