THE EFFECTS OF \( \gamma \)-INTERFERON COMBINED WITH 5-FLUOROURACIL OR 5-FLUORO-2'-DEOXYURIDINE ON PROLIFERATION AND ANTIGEN EXPRESSION IN A PANEL OF HUMAN COLORECTAL CELL LINES

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Gamma-Interferon (IFN-\( \gamma \)) and the antimetabolites 5-fluorouracil (5-FU) and 5-fluoro-2'-deoxyuridine (FUdR) were investigated as individual agents and in combination for their \textit{in vitro} antiproliferative capacity and for their effect on the expression of HLA class-I antigen, carinoembryonic antigen (CEA) and the intracellular tumor-associated antigen CTA-1 in 7 human colorectal cancer cell lines: WiDr, HT29, Colo 205, SW1116, LS174T, SW1398, and LoVo. Growth inhibition by IFN-\( \gamma \) at clinically relevant concentrations (50–100 U/ml) was found in 4/7 cell lines. The cell lines were equally sensitive to 5-FU (IC\textsubscript{50} in a range of 2–10 \( \mu \)M), while sensitivity to FUdR varied considerably (IC\textsubscript{50} in a range of 0.01–90 \( \mu \)M). When 50 U/ml IFN-\( \gamma \) were combined with 5-FU or FUdR, the antiproliferative effects were synergistic in those cell lines with sensitivity to IFN-\( \gamma \) and a single-agent effect in the IFN-\( \gamma \)-insensitive cell lines. IFN-\( \gamma \) was able to enhance the expression of HLA Class I and CEA in 4/7 and 3/7 cell lines, respectively, as measured by flow cytometry. CTA-1 expression could not be enhanced with IFN-\( \gamma \). The expression of the 3 antigens tested was also increased by 5-FU and FUdR. This effect was concentration-dependent in most instances and varied between the individual cell lines. The combination of 50 U/ml IFN-\( \gamma \) with 25% growth-inhibitory concentration of 5-FU or FUdR for each cell line resulted in an additional increase in antigen expression in 4/7 cell lines. No relation was found between the enhancement of antigen expression and the sensitivity to IFN-\( \gamma \) or the anti-metabolites. The enhancement in antigen expression also did not show a relationship with changes in cell-cycle distribution upon exposure to IFN-\( \gamma \) or the anti-metabolites. These results suggest independent mechanisms for the antiproliferative and antigen-enhancing effects of IFN-\( \gamma \), 5-FU and FUdR.

Colorectal cancer is one of the most common malignancies in the western hemisphere and is second to lung cancer as a cause of cancer death (Silverberg, 1986). For the last 30 years 5-fluorouracil (5-FU) has been the chemotherapeutic agent of first choice in the treatment of advanced colorectal cancer, resulting in an objective response rate of approximately 10–20% without a significant effect on overall survival. To improve the therapeutic efficacy of 5-FU in colorectal cancer, nowadays alternative strategies have been identified in the combination of 5-FU with the biochemical modulator leucovorin in advanced disease (Machover, 1989).

Interferons (IFNs) and especially IFN-\( \alpha \) have been investigated both preclinically and in patients for their ability to potentiate the cytotoxic effects of 5-FU. \textit{In vitro} studies have provided evidence of a synergistic antiproliferative action of IFN-\( \alpha \) upon combination with 5-FU against human cancer cell lines (Sato \textit{et al.}, 1984; Elias and Crissman, 1988; Waldler \textit{et al.}, 1989b). Several clinical trials on the efficacy of the combination of IFN-\( \alpha \) and 5-FU have now been performed or are in progress (Waldler \textit{et al.}, 1989a). For IFN-\( \gamma \), which has only recently become available in adequate quantities, \textit{in vitro} studies have also shown a synergistic effect, when this cytokine is combined with 5-FU, against myeloid leukemia and colon cancer cells (Kafka \textit{et al.}, 1989; Le \textit{et al.}, 1984; Elias and Crissman, 1988). Early results of a clinical trial in colorectal cancer with IFN-\( \gamma \) and 5-FU given in combination have shown few responses (Ajani \textit{et al.}, 1989).

In addition to direct anti-tumor toxicity, IFNs also exert an indirect anti-tumor effect by the activation of host defence mechanisms (reviewed by Goldstein and Lazlo, 1986). IFNs have been shown to modulate the expression of antigens of the major histocompatibility complex (MHC) as well as several tumor-associated antigens \textit{in vitro} and \textit{in vivo} (Greiner \textit{et al.}, 1984; Balkwill \textit{et al.}, 1987). The enhancement of tumor-associated antigen expression may be useful to augment antibody-dependent cellular cytotoxicity, or to improve tumor detection with conjugated monoclonal antibodies (MABs) (Rowlinson \textit{et al.}, 1986; Greiner \textit{et al.}, 1987).

For the treatment of advanced colorectal cancer, IFN-\( \gamma \) may be a useful tool to increase the efficacy of 5-FU. Furthermore, the antigen-enhancing capacity of IFN-\( \gamma \) may improve tumor targeting of MABs. Therefore, an \textit{in vitro} analysis of 7 human colorectal cancer cell lines was carried out to determine the antiproliferative capacity of IFN-\( \gamma \), 5-FU and its derivative 5-fluoro-2'-deoxyuridine (FUdR) as single agents and in combination. In addition, apart from IFN-\( \gamma \) each of the antimetabolites was investigated for its effect on the expression of the cell-surface antigens HLA class I and CEA as well as an intracellular antigen CTA-1. HLA class I was selected because it is sensitive to modulation by IFN, whereas CEA and CTA-1 are both targets for radioimmunolocalization studies (Haisma \textit{et al.}, 1990). The combination of IFN-\( \gamma \) at clinically relevant concentrations with 5-FU or FUdR was analyzed in each of the cell lines to determine a possible potentiation in antigen expression.

**MATERIAL AND METHODS**

**Cell lines**

The human colorectal cancer cell lines WiDr (Noguchi \textit{et al.}, 1979), HT29 (Fogh and Trempe, 1975), SW1398 (Rutzky, 1985), SW1116 (Leibovitz \textit{et al.}, 1976), Colo 205 (Semple \textit{et al.}, 1978), LS174T (Tom \textit{et al.}, 1976) and LoVo (Drewinko \textit{et al.}, 1976) were grown in Dulbecco's modified Eagle medium (DMEM) (Flow, Irvine, Scotland) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Flow), 2 mM L-glutamine, 50 IU/ml penicillin and 50 \( \mu \)g/ml streptomycin (Flow) in a humidified atmosphere containing 5% CO\textsubscript{2} at 37°C. Cells were maintained as exponentially growing monolayer cultures by seeding 1 x 10\textsuperscript{6} cells/ml culture medium in a 75-cm\textsuperscript{2} tissue culture flask every 2–3 days.

**Reagents**

Recombinant human IFN-\( \gamma \) with a specific activity of 2–6 x 10\textsuperscript{8} U/mg was kindly provided by Boehringer Ingelheim (Alkmaar, The Netherlands), 5-FU was obtained from Multipharma (Weesp, The Netherlands), and FUdR, propidium iodide and sulphorhamidine B (SRB) were obtained from

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TABLE I – DOUBLING TIME AND ANTIGEN EXPRESSION OF 7 HUMAN COLORECTAL CANCER CELL LINES

<table>
<thead>
<tr>
<th>Cell line</th>
<th>DT (hr)</th>
<th>HLA class-I</th>
<th>CEA</th>
<th>CTA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>WiDr</td>
<td>34.8</td>
<td>8</td>
<td>16</td>
<td>58</td>
</tr>
<tr>
<td>HT29</td>
<td>28.3</td>
<td>8</td>
<td>13</td>
<td>70</td>
</tr>
<tr>
<td>Colo 205</td>
<td>29.0</td>
<td>10</td>
<td>47</td>
<td>60</td>
</tr>
<tr>
<td>SW1116</td>
<td>42.7</td>
<td>13</td>
<td>5</td>
<td>41</td>
</tr>
<tr>
<td>LS174T</td>
<td>28.6</td>
<td>6</td>
<td>22</td>
<td>69</td>
</tr>
<tr>
<td>SW1398</td>
<td>35.2</td>
<td>13</td>
<td>31</td>
<td>54</td>
</tr>
<tr>
<td>LoVo</td>
<td>27.4</td>
<td>1</td>
<td>14</td>
<td>55</td>
</tr>
</tbody>
</table>

1Doubling time in hr. Antigen expression as determined by FACS analysis is defined as the mean fluorescence ratio of cells incubated with the specific antibody and background control cells.

Sigma (St. Louis, MO). Stock solutions of drugs were prepared in a sterile saline solution and stored at room temperature (5-FU and FUDR) or at 4°C (IFN-γ) in aliquots sufficient for individual assays.

Antibodies

The murine MAb W6/32 reactive with a framework determinant of the HLA class-I antigen, the polyclonal rabbit anti-CEA antibody and the FITC-conjugated rabbit antibodies to mouse IgG and human IgM and FITC-conjugated swine antibodies to rabbit IgG were obtained from Dakopatts (Glostrup, Denmark). The human IgM antibody 16.88 directed against the intracellular antigen CTA-1 has been described (Haspel et al., 1985) and was kindly provided by Biotechnology Research Institute (Rockville, MD).

Proliferation assay

Cells were harvested with 0.25% trypsin, 0.2% EDTA (WiDr, HT29, Colo 205) or 2.5% trypsin (SW1116, LS174T, SW1398 and LoVo) to obtain a single-cell suspension and seeded in 96-well tissue-culture plates (50,000 cells/ml, 100 μl/well) and allowed to attach for 24 hr. At day 0, drugs were added in 100 μl culture medium to quadruplicate cultures at doses ranging from 3 or more logs. Control cultures (no growth) were fixed at day 0 with TCA and kept at 4°C to be stained simultaneously with the drug-exposed cultures. For the combination experiments 100 μl culture medium containing a mixture of IFN-γ and 5-FU or FUDR were added, resulting in a final concentration of 50 U/ml IFN-γ and variable concentrations of the anti-metabolites. After 72 hr continuous drug exposure, cells were fixed and cell survival was determined using an SRB assay as described by Skehan et al. (1990). Briefly, the plates were centrifuged and the cells were fixed with 5% ice-cold trichloroacetic acid, washed with water and stained with 0.4% SRB, dissolved in 1% (v/v) acetic acid. After rinsing with 1% acetic acid the plates were air-dried and the bound dye was solubilized with 10 mm unbuffered Tris. The optical density at 540 nm was read. Readings were linear, with cell concentrations between 1,000 and 100,000 cells/well. The percentage of tumor-cell growth for each drug concentration was calculated from at least triplicate cultures as follows:

\[
\text{mean OD exposed cells} - \text{mean OD control} \times 100
\]

For IFN-γ, concentrations of 50–400 U/ml were measured after intramuscular administration of 0.5–2.5 mg/m² (Kurzrock et al., 1985). Cells were defined as sensitive for IFN-γ when 50% growth inhibition (IC50) was observed at these clinically achievable concentrations. A constant concentration of IFN-γ (50 U/ml) was applied in combination experiments with 5-FU and FUDR. The effect of the combination of drugs was considered synergistic when the inhibited fraction of the cells exposed to the combination exceeded the product of the inhibited fractions of the cells exposed to single agents. When this product was equal to or higher than the inhibited fraction of cells exposed to the combination, the respective effects were considered to be additive or antagonistic. Dose-modifying factors of IFN-γ were calculated by dividing the IC50 of the drugs by the IC50 of the drugs in combination with 50 U/ml IFN-γ. All anti-proliferation assays were performed several times and the results show the mean values of at least 3 individual experiments.

Antigen expression

Cells were seeded at 10,000 cells/ml and cultured for 72 hr in the absence or presence of IFN-γ, 5-FU, FUDR or combinations of IFN-γ and each of the antimetabolites. For indirect membrane immunofluorescence, cells were harvested with PBS/EDTA (0.2%), washed twice in PBS, resuspended in PBS containing 1% BSA and 0.02% sodium azide (PBS/BSA) and distributed into 5-ml tubes at 5 × 10⁶ cells/tube. Prior to staining for the cytoplasmic antigen CTA-1, cells were washed twice with PBS and exposed to 100% ice-cold methanol for 15 min. After 2 washes with PBS these cells were also resuspended in PBS/BSA and distributed into 5-ml tubes. The cells were resuspended in 50 μl PBS/BSA containing an excess of first antibody and incubated for 45 min on ice, followed by 3 washes in PBS/BSA. FITC-conjugated second antibody 1:40 diluted in PBS/BSA (50 μl) was added and the cells were again incubated for 45 min on ice. After 3 washes the methanol-fixed cells were resuspended in 500 μl PBS/BSA and the unfixed cells were resuspended in 500 μl 1% paraformaldehyde in PBS. The cells were analyzed for antigen expression by flow cytometry (FACSTARplus, Becton-Dickinson, Eten-Leur, NL). Omission of the first antibody served as background fluorescence control. Mean fluorescence intensity of 10,000 cells was recorded and the background control of individual samples was subtracted. The enhancement of antigen expression was determined by calculating the mean fluorescence ratio of the drug-exposed cells versus the control cells. For each cell line all incubations with the single agents and their combinations were performed in 2 independent experiments and the mean ratios were calculated. A ≥1.5-fold increase in fluorescence intensity was set to define enhancement of antigen expression.

TABLE II – GROWTH INHIBITION BY 5-FU AND FUDR IN THE PRESENCE OR ABSENCE OF IFN-γ

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IFN-γ (U/ml)</th>
<th>5-FU (μM)</th>
<th>FUDR (μM)</th>
<th>IFN-γ (U/ml)</th>
<th>5-FU (μM)</th>
<th>FUDR (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WiDr</td>
<td>50</td>
<td>10</td>
<td>0.2</td>
<td>&lt;0.1</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>HT29</td>
<td>60</td>
<td>10</td>
<td>0.04</td>
<td>0.9 (11)</td>
<td>0.001 (40)</td>
<td></td>
</tr>
<tr>
<td>Colo 205</td>
<td>0</td>
<td>8</td>
<td>0.01</td>
<td>5 (2)</td>
<td>0.007 (2)</td>
<td></td>
</tr>
<tr>
<td>SW1116</td>
<td>0</td>
<td>8</td>
<td>0.4</td>
<td>1 (7)</td>
<td>0.006 (67)</td>
<td></td>
</tr>
<tr>
<td>LS174T</td>
<td>80</td>
<td>7</td>
<td>0.01</td>
<td>0.6 (3)</td>
<td>0.0005 (20)</td>
<td></td>
</tr>
</tbody>
</table>

1Cells were exposed for 72 hr to IFN-γ (50 U/ml) and/or to varying concentrations of the anti-metabolites. 2% of control cell growth at 50 U/ml. IC50 expressed in μM (dose-modifying factor).
Figure 1 – Enhancement of antigen expression by IFN-γ, 5-FU, and FUdR as single agents and in combination. Cells were exposed for 72 hr to IFN-γ (50 U/ml) or one of the anti-metabolites (IC50), or to a combination of IFN-γ (50 U/ml) with 5-FU or FUdR at the IC50. Enhancement of antigen expression as determined by FACS analysis is expressed as the mean fluorescence ratio of drug-exposed cells and control cells.
Cell-cycle analysis

For cell-cycle analysis, cells in logarithmic growth phase were incubated with IFN-γ, 5-FU or FUdR as well as a combination of these drugs for 72 hr. The cells were fixed with 100% methanol and indirect immunofluorescence staining for the Class-I and CEA antigens was performed. After the staining procedure, cells were incubated with RNase (100 μg/ml) for 30 min at room temperature, washed with PBS and resuspended in PBS containing 50 μg/ml propidium iodide. Dual parameter analysis was performed using the FACSTARplus flow cytometer.

RESULTS

Cell lines

The 7 human colorectal cancer cell lines were characterized for the expression of the MHC antigen HLA class I, CEA, and CTA-1. Expression of these antigens as determined by indirect immunofluorescence staining and subsequent FACS analysis revealed different reaction patterns (Table I). Except for LoVo, all lines showed a positive fluorescence for the HLA class-I antigen. All cell lines showed CEA expression, but the intensity of fluorescence varied between the lines. In this respect, LS174T and SW1398 showed the highest expression, WiDr, HT29, and LoVo revealed an intermediate expression, while Colo 205 and SW1116 had the lowest amount of CEA present on the cell membrane. The intracellular antigen CTA-1 detected by 16.88 in methanol-fixed cells was highly expressed in all cell lines.

Proliferation assay

The SRB assay was applied to investigate the proliferation of the colorectal cancer cells exposed continuously to IFN-γ, 5-FU and FUdR. Seeding cell number (5,000 cells/well) and incubation time (72 hr) were chosen after determination of the growth rate of the various cell lines. Doubling times of the cell lines ranged from 27.4 to 42.7 hr (Table I).

Drug concentrations associated with 50% inhibition of tu-

Figure 2 - Enhancement of antigen expression by 5-FU and FUdR. Cells were exposed for 72 hr to 5-FU or FUdR at the IC₅₀ (open columns) or IC₅₀ (filled column). Enhancement of antigen expression as determined by FACS analysis is expressed as the mean fluorescence ratio of drug-exposed cells and control cells.
Table III - Enhancement of Antigen Expression by IFN-γ, 5-FU and FUdR as Single Agent and in Combination

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Drugs</th>
<th>Single drugs at IC50</th>
<th>HLA class I</th>
<th>CEA</th>
<th>CTA-1</th>
<th>+ IFN-γ 50 U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HLA class I</td>
</tr>
<tr>
<td>WiDr</td>
<td>5-FU</td>
<td>2.2</td>
<td>1.4</td>
<td>1.6</td>
<td></td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>FUdR</td>
<td>2.1</td>
<td>1.4</td>
<td>1.5</td>
<td></td>
<td>14.6</td>
</tr>
<tr>
<td>HT29</td>
<td>5-FU</td>
<td>1.3</td>
<td>1.7</td>
<td>1.3</td>
<td></td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>FUdR</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td>7.1</td>
</tr>
<tr>
<td>Colo 205</td>
<td>5-FU</td>
<td>2.6</td>
<td>4.6</td>
<td>2.0</td>
<td></td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>FUdR</td>
<td>5.3</td>
<td>22.6</td>
<td>4.4</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>SW1116</td>
<td>5-FU</td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
<td></td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>FUdR</td>
<td>1.2</td>
<td>1.7</td>
<td>1.1</td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>LS174T</td>
<td>5-FU</td>
<td>1.0</td>
<td>0.9</td>
<td>1.0</td>
<td></td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>FUdR</td>
<td>1.9</td>
<td>1.4</td>
<td>1.5</td>
<td></td>
<td>4.2</td>
</tr>
<tr>
<td>SW1398</td>
<td>5-FU</td>
<td>0.9</td>
<td>1.0</td>
<td>0.6</td>
<td></td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>FUdR</td>
<td>1.1</td>
<td>0.9</td>
<td>0.9</td>
<td></td>
<td>1.3</td>
</tr>
<tr>
<td>LoVo</td>
<td>5-FU</td>
<td>neg</td>
<td>2.8</td>
<td>2.7</td>
<td></td>
<td>neg</td>
</tr>
<tr>
<td></td>
<td>FUdR</td>
<td>neg</td>
<td>2.8</td>
<td>2.8</td>
<td></td>
<td>neg</td>
</tr>
</tbody>
</table>

1 Cells were exposed for 72 hr to IFN-γ (50 U/ml) and/or to one of the anti-metabolites at the IC50. Enhancement of antigen expression as determined by FACS analysis is expressed as the mean fluorescence ratio of drug-exposed cells and control cells.
phase. No change in cell-cycle distribution could be detected after incubation with 50 U/ml IFN-γ for 72 hr. Antigen expression of cells exposed to IFN-γ (50 U/ml) was enhanced but showed the same distribution through the different stages of the cell cycle. Exposure to 5-FU or FUDR at the IC<sub>50</sub> and IC<sub>100</sub> resulted in an increased number of cells in S-phase but antigen expression was similarly enhanced in all stages of the cell cycle. In this respect, a relation between antigen expression and cell-cycle distribution could not be demonstrated.

**DISCUSSION**

In a panel of 7 human colorectal cancer cell lines with different growth rates and antigen expression, we measured the in vitro effects of IFN-γ, 5-FU and FUDR on cell growth and antigen expression. The anti-proliferative effect of the combination of IFN-γ with 5-FU or FUDR was increased in those cell lines (4/7) that were sensitive to IFN-γ. The expression of the 3 different antigens studied could be increased in a number of lines by IFN-γ, 5-FU or FUDR, while combination of the cytokine with each of the anti-metabolites showed synergistic enhancement of the antigens in 4/7 cell lines. No relationship could be detected between anti-proliferative capacity and the antigen-enhancing effects of the drugs.

We were interested in the mechanism of IFN-γ in its modulation of the cytostatic effects of 5-FU and FUDR as in vitro a synergism between IFN-γ and the antimetabolites has been indicated (Le et al., 1984; Elias and Crissman, 1988; Kafka et
γ-INTERFERON, 5-FLUOROURACIL AND 5-FLUORO-2'-DEOXYURIDINE

Several groups have reported an enhanced MAb targeting to human tumor xenografts grown in nude mice due to increased antigen expression following administration of human IFNs (Rowlinson et al., 1986; Greiner et al., 1987; Matsui et al., 1988). We therefore investigated a possible effect on antigen expression by IFN-γ for several antigens in the 7 colon cancer cell lines. We found enhanced expression of HLA class I and CEA in 4 and 3 cell lines, respectively. CTA-1 expression was not increased after IFN-γ exposure. The sensitivity to IFN-γ and the enhancement of antigen expression by IFN-γ in our cell lines were not related. The enhancement or induction of antigen expression by IFN-γ has been described and appears to be related to the number of IFN-γ receptors per cell (Ucer et al., 1985; Berkovic et al., 1986). WiDr and Colo 205 cells, known to express a high number of IFN-γ receptors (Ucer et al., 1985), and HT29 indeed showed the most marked enhancement of antigen expression in our experiments.

Apart from IFN-γ, we studied the effects on antigen expression of subtoxic concentrations of 5-FU and FUDR. 5-FU and FUDR could indeed increase the expression of HLA class I, CEA and CTA-1, but the degree varied between the lines. Combinations of IFN-γ with the anti-metabolites resulted in additional enhancement of these antigens in 4/7 cell lines. There was no correlation between the constitutive antigen expression and the sensitivity to modulation of antigen expression. The antigen-enhancing effect of the anti-metabolites was not related to the drug sensitivity of the cell lines, neither was there a relation with the cell-cycle distribution after drug treatment.

Several mechanisms may be involved in the interaction between IFN-γ and 5-FU or FUDr with respect to the inhibition of cell proliferation and antigen expression. The increased conversion of 5-FU to FUDr to 5-fluorodeoxyuridylate (FdUMP) by IFN-γ (Elias and Sandoval, 1989), will result in enhanced inhibition of the enzyme thymidylate synthase (TS) and subsequently in the inhibition of DNA synthesis. One of the proposed mechanisms for cell kill is "thymineless death" (Elias and Sandoval, 1989). From our results it is not yet clear whether the increased antigen expression is the result of a non-specific effect of 5-FU or FUDr on protein synthesis in general or a specific effect on antigen expression. The latter might be related to a specific effect of 5-FU on DNA synthesis and subsequently on DNA transcription leading to enhanced expression of certain genes. Another explanation for the effect of 5-FU on antigen expression may be found in the incorporation of 5-FU into RNA. As a consequence, RNA processing may be disturbed, and cause alterations in the expression of several proteins (Will et al., 1986; Iwata et al., 1986). A third explanation to be considered is the possible interference of 5-FU with post-transcriptional events, such as glycosylation of protein, which may alter the expression of several antigens (Peters et al., 1990).

Because of different responses obtained with IFN-γ and the antimitobolites in human colorectal cancer cell lines with respect to drug sensitivity and the extent of antigen expression, a heterogeneous response can be anticipated in colorectal cancer patients. Nonetheless, our data support the design of clinical trials of IFN-γ combined with 5-FU or FUDR. Improved treatment results may be expected in subsets of patients with IFN-γ-sensitive tumors. Moreover, combination of IFN-γ with 5-FU or FUDR may be valuable for the improvement of tumor localization and radioimmunotherapy with MAbs directed to tumor-associated antigens, such as CEA in colorectal cancer patients.

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