SMS 201-995 enhances S-phase block induced by 5-fluorouracil in a human colorectal cancer cell line

Dražen Massari, Zlatko Trobonjač, Daniel Rukavina and Biserka Radošević-Stašić

The action of the somatostatin analog SMS-201.995 (SMS) was tested in monotherapy and in combined therapy with the cytotoxic agent 5-fluorouracil (5-FU) on cell cycle kinetics of the human colon cancer cell line WiDr, expressing a mutant p53 (mp53). The data, obtained by flow cytometric DNA analysis, showed that SMS at 0.2 μg/ml increased apoptosis, augmenting the proportion of cells with subdiploid DNA content by 65 and 48% after 3 and 6 h, respectively. In cultures lasting 24 and 36 h, it also decreased the percentages of cells in G_0/G_1 phase by 22.9 and 14.3%; whereas at a dose of 0.1 μg/ml, SMS decreased the percentage of cells in G_2/M by 14.3%. In contrast to SMS, 5-FU (0.1 μg/ml) augmented the apoptosis at 12 h, and markedly increased the fraction of cells in S phase, increasing its value from 24 and 72 h by 108 and 234%, respectively, in comparison to the control. The most evident finding after the combination of SMS (0.2 μg/ml) and 5-FU (0.1 μg/ml) was a potentiation of 5-FU-induced S-phase block by a further 7.9, 12.9 and 42.1% at 24, 36 and 72 h, respectively. Treatment with 5-FU also upregulated HLA class I expression of the cancer cells. In this sense, SMS was less effective and when given in combination with 5-FU did not change the effects induced by 5-FU. The data emphasize that SMS exhibits pro-apoptotic and anti-proliferative effects, which in proper dose combinations might enhance the effects of 5-FU on human colorectal cancer cells expressing mp53. *Anti-Cancer Drugs* 16:989–996 © 2005 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2005, **16**:989–996

Keywords: cell cycle kinetics, HLA class I expression, human colorectal cell line WiDr, SMS 201-995 (octreotide)

Department of Physiology and Immunology, Medical School, University of Rijeka, Rijeka, Croatia.

Sponsorship: This work was supported by a grant from the Croatian Ministry of Science (project 0062018).

Correspondence to B. Radošević-Stašić, Department of Physiology and Immunology, Medical School, University of Rijeka, B. Branchetta 22, 51 000 Rijeka, Croatia.
Tel: +38551651150; fax: +38551675699;
e-mail: biserr@medri.hr

Received 2 May 2005 **Revised form accepted** 8 July 2005

Introduction

Colorectal cancer (CRC) is the second to the fourth most common cancer in industrialized countries, affecting more than 145 000 new people in the US annually [1,2]. The prognosis for patients is heavily dependent on stage at diagnosis and although the 5-year survival is over 90% for Duke's A, it is only 5% for Duke's D, indicating incompetence of therapy.

The most commonly administered chemotherapy agents is 5-fluorouracil (5-FU) used in combination with leucovorin or other chemotherapeutics [3–5]; however, the great polymorphism in drug-metabolizing enzymes, as well as the high-penetrance mutations in several other genes, might induce an overexpression or underexpression of drug targets leading to resistance or toxicity to standard chemotherapy regimens. This also points to the necessity of a polygenic approach to pharmacogenetic studies and individualization of therapy [6]. The combinations of agents affecting different pathways in tumor growth might, therefore, present some considerable improvements in the effects of therapeutic strategies.

In this sense there is a lot of controversy regarding the anti-neoplastic effects of somatostatin and its analogs used in monotherapy or in combined therapy with other cytotoxic agents or other hormones [7–12]. Generally, it was concluded that apart from some notable exceptions, somatostatin analog therapy was very disappointing in the management of advanced malignancy [11], although it was reported that a long-acting somatostatin analog SMS 201-995 (SMS; octreotide, sandostatin) might reduce the size of endocrine tumors, such as growth hormone (GH)-producing pituitary adenomas, thyroid-stimulating hormone-secreting adenomas, carcinoid tumors and vasoactive intestinal peptide-secreting adenoma [7–10,12–14]. Some improvements were also observed in the management of solid tumors [15,16], as well as of colorectal micro-metastases in the liver [17]. Similarly, it was noticed that somatostatin and its analogs exhibit anti-proliferative effects on several tumor cell lines, using five G-protein-coupled receptors and diverse signal transduction pathways, including adenylate cycles, phospholipase C-β, phospholipase A_2, guanylate cyclase, ionic conductance channels and tyrosine phosphatase [18–23].

0959-4973

As our previous findings showed that SMS has marked anti-proliferative and immunomodulatory properties [24,25], in the present study we attempted to analyze its effects in monotherapy and in combined therapy with standard chemotherapeutic 5-FU on the human CRC cell line WiDr, which like most human CRCs [1] expresses a mutant p53 (mp53). The data obtained by the evaluation of cell cycle kinetics revealed that SMS alone has pro-apoptotic and anti-proliferative effects, as well as that SMS in combination with 5-FU might potentiate the 5-FU-induced arrest of cells in S phase of the cycle. This suggested that proper timing and combination of these drugs might improve the anti-neoplastic therapy. However, in contrast to 5-FU, SMS was a less-effective inducer of HLA class I expression on WiDr cells.

Material and methods

Cell culture and cell growth study

Human colorectal adenocarcinoma cell lines WiDr with mp53 (ECACC 85111501) was obtained from ATCC (Rockville, Maryland). The cells were grown in RPMI 1640 (Life Technologies, Grand Island, New York, USA) supplemented with fetal bovine serum [10% (v/v); Hyclone, Logan, Utah, USA], glutamine (2 mM), penicillin (100 000 U/l) and streptomycin (100 mg/l). Long-term cell cultures were maintained at 37°C in a humid atmosphere of 5% CO_2/95% air. The medium was replaced every second day and passaging was performed by exposing the cells to 0.1% trypsin for 10 min.

For the cell growth study, floating and trypsinized adherent cells were collected, washed 3 times in RPMI and centrifuged at 2500 *g* for 5 min at 4°C. Cells were counted using a hemocytometer and 0.5×10^6 cells/2 ml medium were cultivated in 24 well dishes and treated with 5-FU (Pliva, Zagreb, Croatia) and/or SMS (Novartis, Basel, Switzerland) in different concentrations and combinations.

Flow cytometric cell cycle and DNA fragmentation analysis

In cultures lasting 3, 6, 12, 24, 36, 48 and 72 h, cell cycle analysis was undertaken using a flow cytometer together with CellFit software (Becton Dickinson, Mountain View, California, USA) and CellQuest software (Macintosh, Quadra 650). For this purpose, floating and trypsinized adherent cells were collected, suspended in PBS (pH 7.3), fixed with 70% (v/v) ethanol and stained with propidium iodide (PI; Sigma, St Louis, Missouri, USA) staining solution (0.05 mg/ml PI, PBS, pH 7.4 with 1% glucose, 0.1 mg/ml RNase A) for 1 h at room temperature in the dark. Cell cycle analysis was performed on a FACScan flow cytometer (Becton Dickinson), collecting data on 10 000 cells from each sample. Debris was excluded by selective forward versus side scatter gating. The percentage of cells with subdiploid DNA was quantified by the CellFit software by plotting intensity of fluorescence versus number of cells. The percentage of cells located to the left of the $G_{0/1}$ peak, diagnostic of hypodiploid cells that have lost DNA, was taken as the percentage of apoptotic cells [[26]].

Determination of major histocompatibility antigen class (HLA) I expression

HLA I antigen expression was quantified using flow cytometry. The monoclonal mouse antibody W6/32 against the monomorphic region of human HLA (IgG2a,κ) was obtained from Dako (Hamburg, Germany).

Statistical analysis

Statistical analysis was performed by Student's *t*-test for unpaired samples or by the Mann–Whitney *U*-test, using the Sigma Plot (Jandel Scientific, Corte Madera, California, USA) scientific graphing system, version 6.10. The differences were considered significant for $P < 0.05$. Data are reported as means ± SEM, unless otherwise noted.

Results

DNA content of cells was analyzed in cultures lasting 3, 6, 12, 36, 48 and 72 h after monotherapy with SMS or 5-FU, as well as after the combination of these two agents. The data were compared with the findings obtained in cells maintained in medium only. Each experiment was performed a minimum of 4 times, with one sample of each concentration compared with a control sample on each occasion.

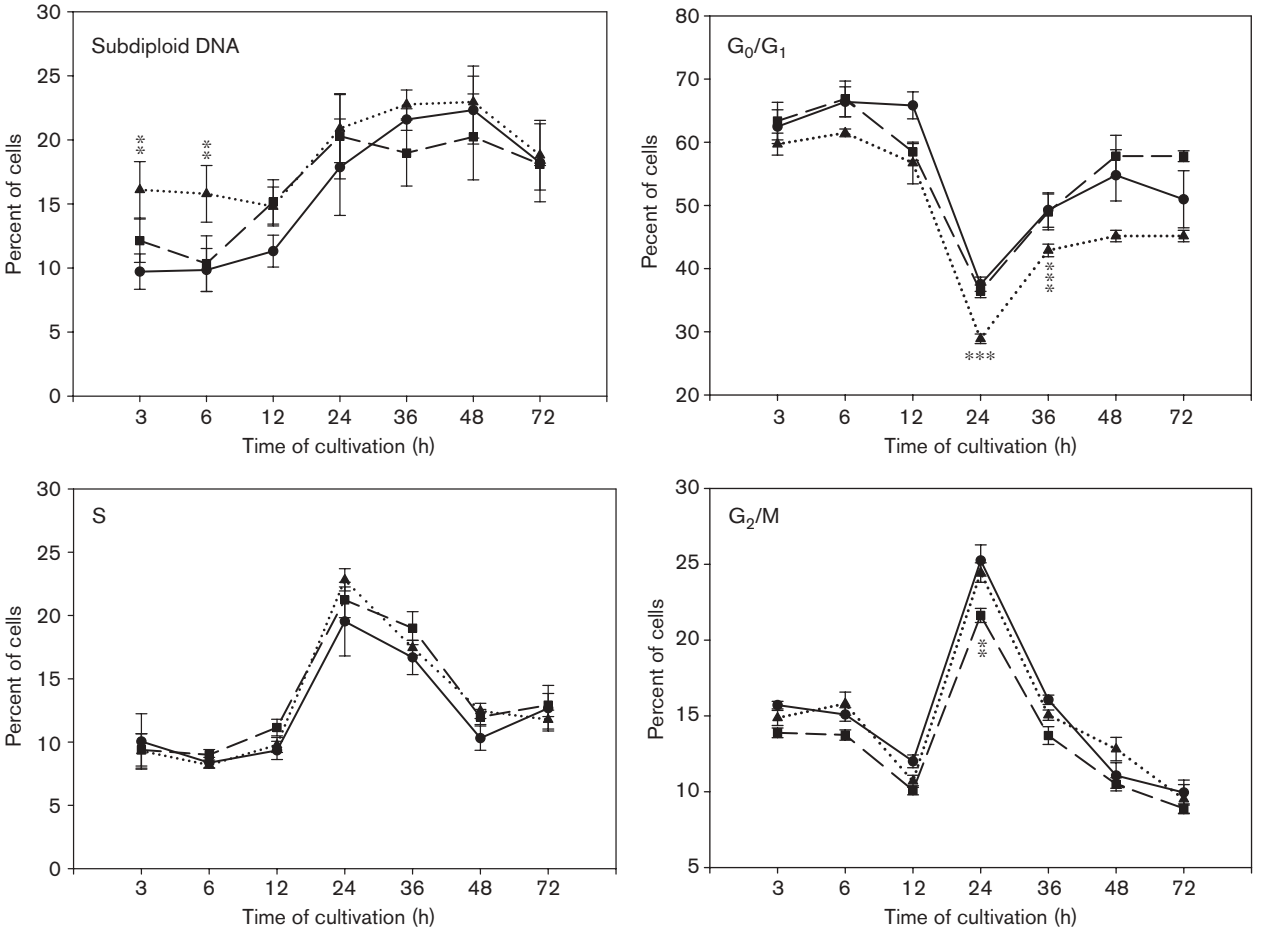
Cell cycle kinetics of the human CRC cell line WiDr after monotherapy with SMS

Treatment with SMS at a dose of 0.2 μg/ml stimulated apoptosis after 3 and 6 h, increasing the proportion of cells with subdiploid DNA content by 65 and 48%, respectively (from 9.7 ± 1.3% in the control culture to 16.01 ± 2.2 and 14.5 ± 3.4% at 3 and 6 h. respectively; Fig. 1; $P < 0.01$). Furthermore, at 24 and 36 h, SMS (0.2 μg/ml) also decreased the percentages of cells in G_0/G_1 phase by 22.9 and 14.3% ($P < 0.0001$), respectively, while at a dose of 0.1 μg/ml SMS, it decreased the percentage of cells in G_2/M phase by 14.3% ($P < 0.003$), suggesting its inhibitory effect on mitotic cells.

Cell cycle kinetics of the human CRC cell line WiDr after monotherapy with 5-FU

In contrast to SMS, treatment with 5-FU (Fig. 2) at a dose of 0.1 μg/ml increased the percentage of subdiploid cells at 12 and 72 h by 96 and 107% (from 11.3 ± 1.2 to 22.2 ± 4.1%, $P < 0.01$ and from 18.2 ± 3.0 to 37.7 ± %, $P < 0.05$, respectively), and provoked a marked arrest of cells in S phase of the cycle, increasing its value at 24–72 h by 108, 123, 234 and 81.7%, respectively, in comparison with values found in the untreated cultures (i.e. from 19.5 ± 2.7 to 40.7 ± 1.3%, from 16.6 ± 1.3 to 37.1 ± 1.0%, 10.3 ± 0.9 to 34.4 ± 1.8%, $P < 0.0001$ and

Fig. 1



Cell cycle distribution of human colorectal WiDr cancer cells after treatment with SMS at (squares) 0.1 and (triangles) 0.2 μg/ml in comparison with cell cycle distribution of untreated cells (control; circles). Cells were grown in culture media recommended by the ATCC, and analyses were performed on a flow cytometer using CellFit and CellQuest Software as described. Each experiment was performed a minimum of 4 times with one sample of each concentration compared to a control sample on each occasion. Data are means ± SEM. $^{**}P<0.01$; $^{***}P<0.001$.

from 12.6 ± 1.8 to 22.9 ± 1.1%, $P < 0.01$, respectively). In contrast, higher doses of 5-FU (1 and 10 μg/ml) arrested more cells in the G_0/G_1 phase (at 24 h; $P < 0.0002$).

The effects of combined therapy with 5-FU and SMS

The combination of 5-FU and SMS was tested in various dose combinations (not shown). The most evident changes were obtained by combining SMS (0.2 μg/ml) and 5-FU (0.1 μg/ml) in cultures lasting 24, 36 and 48 h, where SMS in comparison with the effect of 5-FU alone potentiated and prolonged the 5-FU-induced S phase block, increasing this fraction of cells by an additional 7.9% (from 40.7 ± 1.3 to 43.9 ± 1.0%; $P < 0.05$), 12.9% (from 37.1 ± 1.0 to 41.9 ± 1.0%; $P < 0.003$) and 42.1% (from 34.4 ± 1.8 to 48.8 ± 1.8.0%, $P < 0.002$), respectively (Fig. 3). Simultaneously, in the interval between 36 and 48 h, the addition of SMS decreased the proportion of cells in G_0/G_1 as well as in the G_2/M phase (after 24 and 36 h). The same combination also enhanced the percentage of apoptotic cells, increasing significantly the proportion of cells with subdiploid DNA at 36 h by *32%* (from 22.7 ± 1.1 to 29.97 ± 1.7, $P < 0.002$) in comparison with cultures treated with SMS alone (Fig. 3).

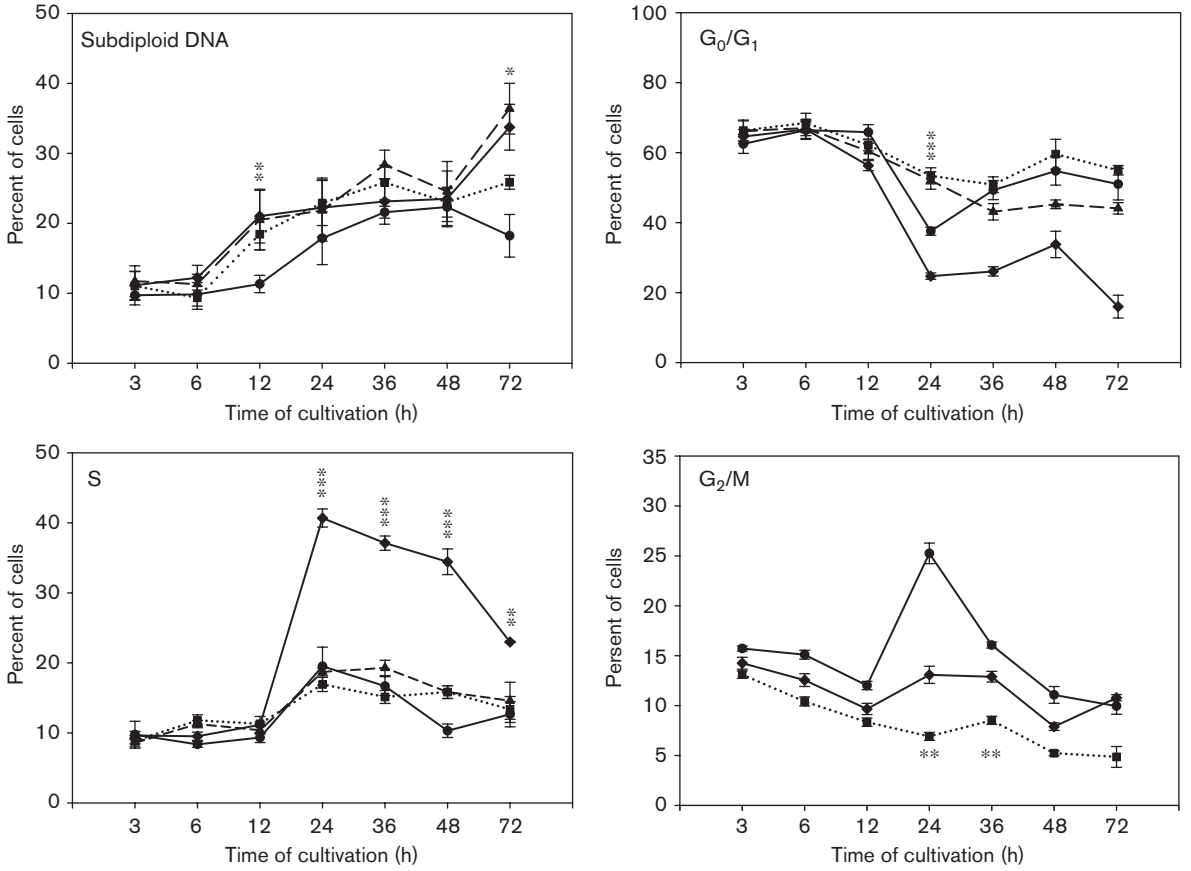
Modulation of HLA class I expression on the human CRC cell line WiDr by 5-FU and SMS

Cancer cells treated with 5-FU markedly upregulated the expression of membrane-associated HLA class I, increasing after 72 h (at a dose of 0.1 μg/ml) the mean fluorescence intensity (MFI) to 3.73 (Fig. 4). In this sense, SMS was less effective, increasing the MFI to 2.69 at a concentration of 1 μg/ml. Combined therapy did not produce larger effects than 5-FU alone.

Discussion

The data show that SMS alone has pro-apoptotic and anti-proliferative properties, which in certain combinations with 5-FU may enhance the anti-neoplastic effects

Fig. 2



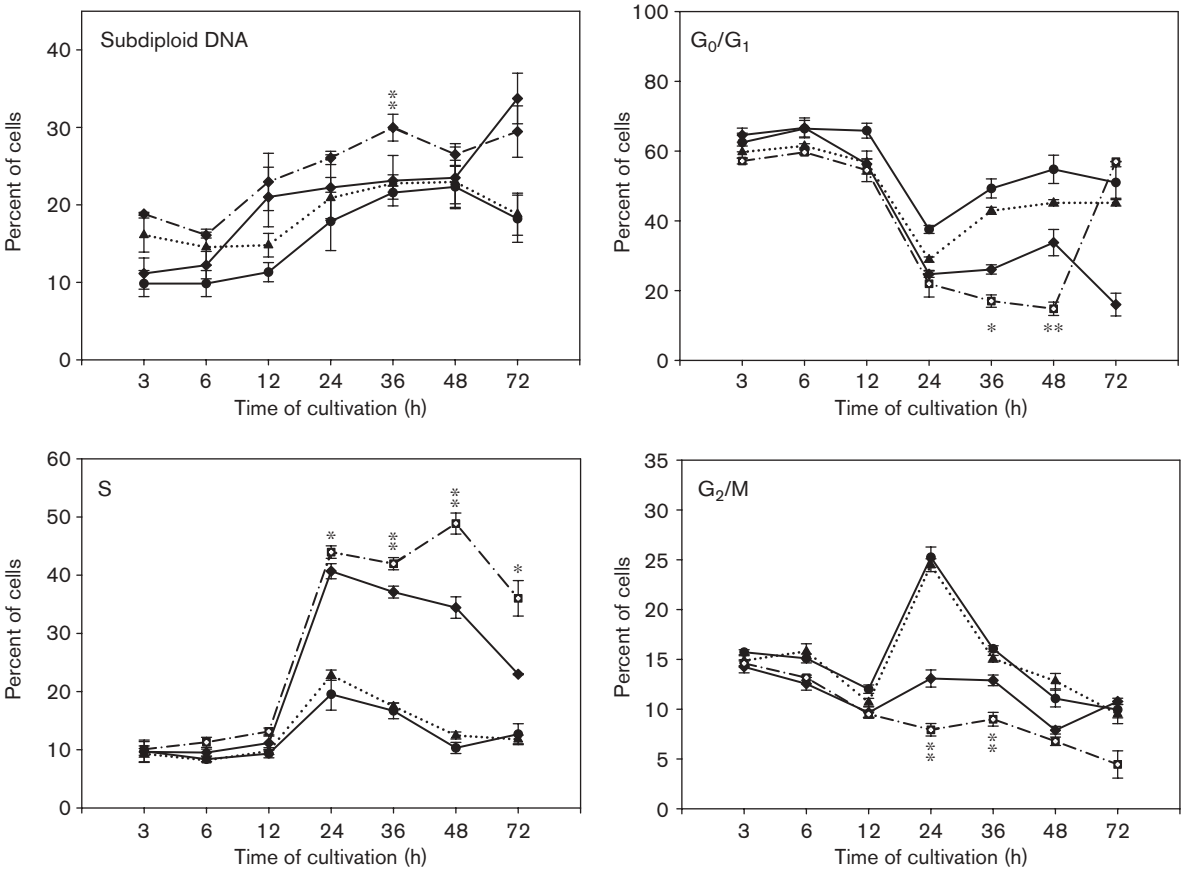
Cell cycle distribution of human colorectal WiDr cancer cells after treatment with various doses of 5-FU [(diamonds) 0.1, (triangles) 1 and (squares) 10 μg/ml) in comparison with the cell cycle distribution of untreated cells (control; circles). Data are means ± SEM. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

of 5-FU (Figs 1 and 3). This is of particular interest, because the investigations were performed on the human CRC line WiDr, which expresses abnormal p53 protein with a missense point mutation, similar to more than 75% of human colon carcinomas [1,27] in which these mutations might also determine the sensitivity to 5-FU and other chemotherapeutics [28].

The results are consistent with previously published reports [22,23,29–33], but emphasize again that the findings in this field are dependent on type of tumor cells, conditions of cultivation, as well as on doses and timing of drug combinations. This indicates that the final outcome depends on the interaction of drugs with diverse signal transduction pathways, activated by SMS and 5-FU.

Anti-proliferative effects of somatostatin are mediated either indirectly, through inhibition of various mitogenic factors, or by direct receptor-mediated actions on the cells [7–10,34]. Examples of the former include suppression of trophic hormones, such as GH, prolactin and gastrointestinal hormones, and suppression of paracrine or autocrine activity, such as epidermal growth factor (EGF) and insulin-like growth factors [35]. The direct effects of somatostatin are mediated through a family of five somatostatin receptors (sst1–sst5) which regulate diverse signal transduction pathways, including adenylate cyclase, Ca^{2+} and K^+ channels, phospholipase C-β, phospholipase A_2, guanylate cyclase, serine/threonine phosphatases, and tyrosine phosphatases [8,10,18–23,34,35]. Native somatostatin binds to all five subtypes similarly, but a somatostatin analog, SMS, binds with high affinity to the sst2 and sst5 receptor subtypes, and with a moderate affinity to sst3, suggesting that only the tumors expressing these types of receptors would be sensitive to therapy with SMS. Thus, good experience has been obtained with SMS therapy in gastrointestinal neuroendocrine carcinomas [36,37]. This is in contrast to advanced pancreatic cancer [38] or CRC, both of which minimally express SMS-binding sites [8]. However, even in the later conditions, it was noticed that SMS might

Fig. 3



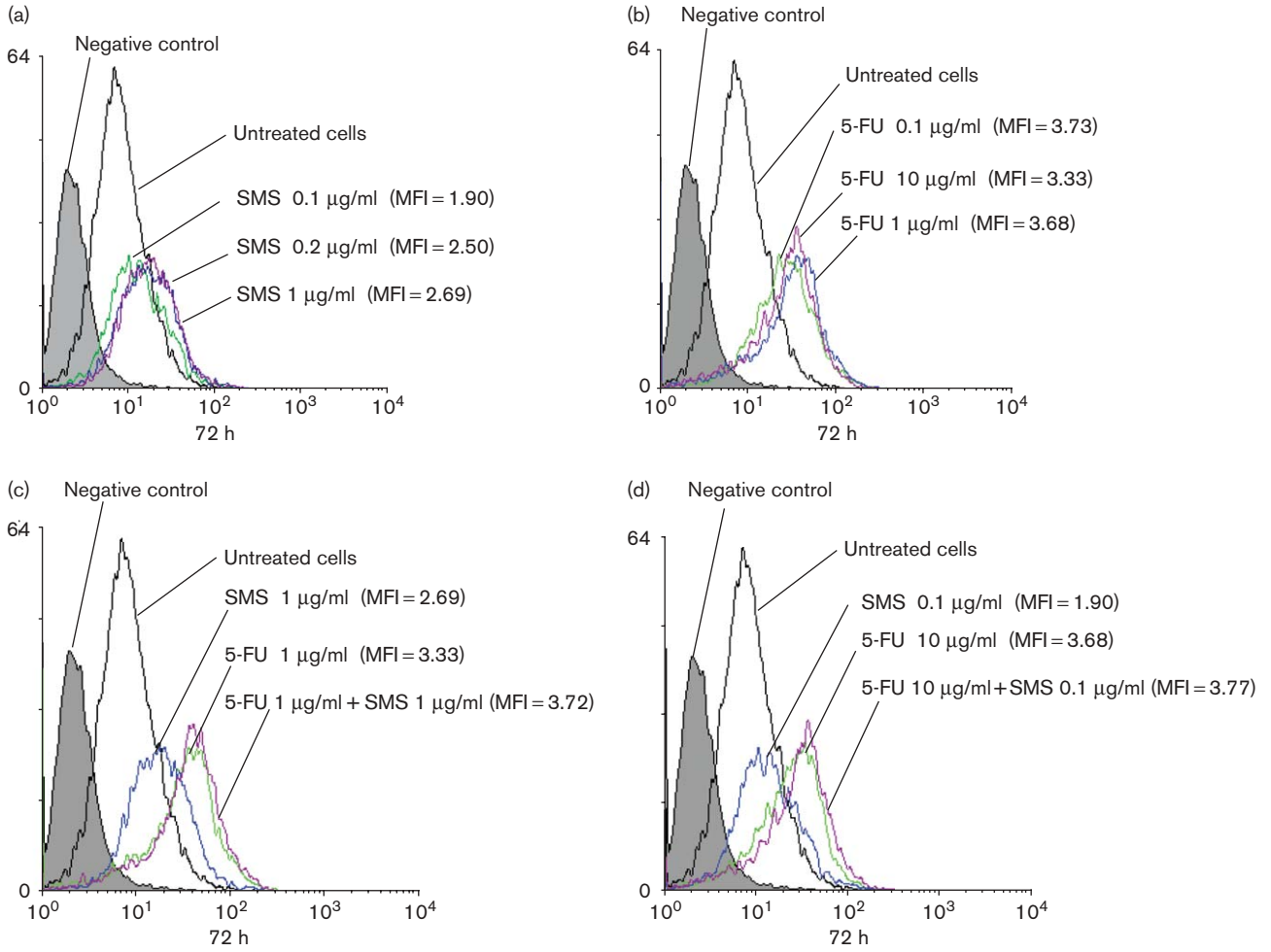
Cell cycle dynamics of human colorectal WiDr cancer cells after monotherapy (triangles and diamonds, respectively) and/or combined (open squares) therapy with SMS (0.2 μg/ml) and 5-FU (0.1 μg/ml) in comparison with the cell cycle distribution of untreated cells (control; circles). Data are means ± SEM. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

have some indirect influence, since somatostatin receptors might be locally upregulated by blood vessels around the tumor, resulting in inhibition of angiogenesis in neoplastic tissue [8,39].

Additionally, SMS has been shown to inhibit cell proliferation of several human colorectal cells lines and to suppress the growth of tumors derived from these lines, both *in vitro* and *in vivo* [23,40–44]. It reduced the DNA and protein content in these tumors [43], and inhibited carcinoembryonic antigen secretion of human colon cancer lines [45] and of primary rectal carcinoma in patients [46]. Furthermore, treatment with SMS also decreased the mitogenic effect of EGF [23] and depressed the growth-promoting effect of gastrin on the transplanted carcinoma, suggesting that SMS might block the effects of endogenous gastrin, which can promote the growth of some CRCs by regulating the expression of oncogenes c-*myc* and c-*fos* [47]. Occasionally, however, in-vitro effects of SMS have not been obtained, due to the very low expression of SST proteins or due to the loss of somatostatin receptors in previous passages of cell lines [48].

More evidence about the effects of somatostatin on the cell cycle machinery has been obtained from experiments on heterologous cells, transfected with individual SST receptor subtypes. Recent data elucidated that $G_{i/0}$-protein-coupled sst2 receptor inhibits cell proliferation especially by activation of a tyrosine phosphatases SHP-1 and SHP-2, leading to induction of the cyclin-dependent kinase (CDK) inhibitor $p27^{kip1}$, as a consequence of a strong and transient stimulation of ERK2 signaling via a SHP-1–SHP-2–PI3K/Ras–Rap1/B-Raf/MEK1/2 pathway [49]. In Chinese hamster ovary DG44 cells stably expressing sst2, the somatostatin analog RC-160 has promoted G_1 cell cycle arrest of insulin-treated cells [50], similar to somatostatin 14 and SMS on proliferating GH3 rat pituitary tumor [51]. However, apart from these cytostatic and anti-proliferative effects, SMS might have also p53-dependent and -independent pro-apoptotic activities, mediated by the activation of sst3 and sst2

Fig. 4



Flow cytometric analysis of HLA class I expression on human colorectal WiDr cancer cells after monotherapy and/or combined therapy with SMS and 5-FU. (a) SMS alone at 72 h, (b) 5-FU alone at 72 h, (c) combined therapy with 5-FU (1 μg/ml) + SMS (1 μg/ml) and (d) combined therapy with 5-FU (10 μg/ml) + SMS (0.1 μg/ml). Histograms represent the MFI obtained upon staining with monoclonal mouse antibody W6/32 against the monomorphic region of human HLA (IgG2a,κ) or with isotypic control.

receptors, respectively [52]. Consistent with the later hypothesis is that SMS in monotherapy induces an acute apoptosis visible after 3 and 6 h of treatment (Fig. 1). G_0/G_1 block, however, was not seen, probably due to the presence of mp53 in the WiDr cell line; instead, the remaining cells progressed to the S and G_2/M phase, where a greater inhibitory effect was seen after 24 h. The later, however, was obtained only for a dose of 0.1 μg/ml ($P < 0.003$), which in contrast to a dose of 0.2 μg/ml did not show any pro-apoptotic effect (Fig. 1).

In combination with 5-FU, SMS, markedly potentiated the 5-FU-induced accumulation of cells in S phase, indicating interference with downstream effectors of 5-FU-activated mechanisms. In this sense, our data are in agreement with previously reported findings which showed that combinations of octreotide and 5-FU might result either in additive or, at high concentrations of the chemotherapeutic agent, in synergistic interactions [31,32]. The mechanisms of this interference are still unclear.

Generally it is held that the anti-metabolite 5-FU, which is predominantly used for the treatment of epithelial cancers, possesses a dual mechanism of action that depends on the concentration of the drug and the cellular characteristics of target cells [3–5]. In the first mechanism, 5-FU inhibits thymidylate synthase (TS) after extensive metabolism, where the active metabolite FdUMP forms a covalent ternary complex with the enzyme and the reduced folate cofactor, 5,10-methylenetetrahydrofolate, used in the normal catalytic reaction. The induction of a thymine-less state leads to DNA damage and subsequent death. The second mechanism of

5-FU cytotoxicity is that following metabolism of the drug to ribonucleotides with subsequent incorporation of FUTP into RNA, resulting in aberrant processing of RNA species [53]. The effects are highly dependent on p53, since in the presence of a wild-type *p53* gene both types of cytotoxicities induce Fas-mediated acute apoptosis in several human colon carcinoma cell lines, while in the presence of mutant *p53*, cells undergo prolonged S-phase arrest followed by delayed apoptosis [53–56].

Our data, showing that in the WiDr colon cell line with mp53, lower concentrations of 5-FU (0.1 μg/ml) block the cells in S phase, also inducing late apoptosis (Fig. 2), seems to support some of the previous findings. The results, however, only permit us to speculate about the mechanisms by which SMS given in combination with 5-FU (5-FU 0.1 μg/ml + SMS 0.2 μg/ml) enhanced the 5-FU-induced S-phase block and translocated the apoptosis to an earlier time interval (Fig. 3). There is the possibility that SMS retarded the degradation of 5-FU or activated some pathways that induced RNA cytotoxicity, but proof is lacking, although it is known that in somatostatin-induced apoptosis the activation of membrane-associated SHP-1 induces the intracellular acidification which precedes the mitochondrial dysfunction [57], as well as that treatment with SMS increases the expression of the p53 tumor suppressor protein breakdown product [58]. Furthermore, since the activation of sst2 somatostatin receptors prevents growth factor-induced cell proliferation through activation of the tyrosine phosphatase SHP-1 leading to induction of the CDK inhibitor $p27^{kip1}$ [50], there is also the possibility that SMS inactivated CDK/cyclin complexes and blocked the further cell cycle progression.

Our data also show that both 5-FU and SMS given as monotherapy may increase the surface expression of HLA class I on the human colorectal line WiDr (Fig. 4), suggesting that after in-vivo application they probably contribute to abrogation of the failure of T lymphocyte recognition during an immune response against a tumor, which is often seen in cancer patients. However, combinations of 5-FU and SMS did not give an additive effect, and the mechanisms are unclear, although the data are in agreement with previously published evidence showing that 5-FU may stabilize HLA class I mRNAs, leading to their accumulation [59], as well as with our observation that SMS may upregulate the expression of $H\text{-}2^{d}$ antigens on YAC-a tumor cells line after prolonged in-vitro treatment (unpublished data).

In conclusion, our results obtained on the human colon cancer cell line WiDr with mp53 show that proper timing and dose combinations of 5-FU and SMS might result in enhanced growth arrest and pro-apoptotic effects, suggesting that they might be useful in the therapy of human CRC.

References

1 de la Chapelle A. Genetic predisposition to colorectal cancer. *Nat Rev Cancer* 2004; **4**:769–780.
2 Johns LE, Houlston RS. A systematic review and meta-analysis of familial colorectal cancer risk. *Am J Gastroenterol* 2001; **96**:2992–3003.
3 Parker WB, Cheng YC. Metabolism and mechanism of action of 5-fluorouracil. *Pharmacol Ther* 1990; **48**:381–395.
4 Yoshikawa R, Kusunoki M, Yanagi H, Noda M, Furuyama JI, Yamamura T, *et al.* Dual antitumor effects of 5-fluorouracil on the cell cycle in colorectal carcinoma cells: a novel target mechanism concept for pharmacokinetic modulating chemotherapy. *Cancer Res* 2001; **61**:1029–1037.
5 Backus HH, Pinedo HM, Wouters D, Kuiper CM, Jansen G, van Groeningen CJ, *et al.* Differences in the induction of DNA damage, cell cycle arrest, and cell death by 5-fluorouracil and antifolates. *Oncol Res* 2000; **12**:231–239.
6 Evans WE, McLeod HL. Pharmacogenomics – drug disposition, drug targets, and side effects. *N Engl J Med* 2003; **348**:538–549.
7 Jenkins SA, Kynaston HG, Davies ND, Baxter JN, Nott DM. Somatostatin analogs in oncology: a look to the future. *Chemotherapy* 2001; **47**: 162–196.
8 Reubi JC. Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocr Rev* 2003; **24**:389–427.
9 Bousquet C, Guillermet J, Vernejoul F, Lahlou H, Buscail L, Susini C. Somatostatin receptors and regulation of cell proliferation. *Dig Liver Dis* 2004; **36**:S2–S7.
10 Hofland LJ, Lamberts SWJ. The pathophysiological consequences of somatostatin receptor internalization and resistance. *Endocr Rev* 2003; **24**:28–47.
11 Goldberg RM, Moertel CG, Wieand HS, Krook JE, Schutt AJ, Veeder MH, *et al.* A phase III evaluation of a somatostatin analogue (octreotide) in the treatment of patients with asymptomatic advanced colon carcinoma. North Central Cancer Treatment Group and the Mayo Clinic. *Cancer* 1995; **76**:961–966.
12 Morris DL. A phase III evaluation of a somatostatin analogue (octreotide) in the treatment of patients with asymptomatic advanced colon carcinoma. *Cancer* 1996; **77**:1956–1957.
13 Lee MT, Liebow C, Kamer AR, Schally AV. Effects of epidermal growth factor and analogues of luteinizing hormone-releasing hormone and somatostatin on phosphorylation and dephosphorylation of tyrosine residues of specific protein substrates in various tumors. *Proc Natl Acad Sci USA* 1991; **88**:1656–1660.
14 Shimon I, Yan X, Taylor JE, Weiss MH, Culler MD, Melmed S. Somatostatin receptor (SSTR) subtype-selective analogues differentially suppress *in vitro* growth hormone and prolactin in human pituitary adenomas. Novel potential therapy for functional pituitary tumors. *J Clin Invest* 1997; **100**:2386–2392.
15 Wang CH, Tang CW, Liu CL, Tang LP. Inhibitory effect of octreotide on gastric cancer growth via MAPK pathway. *World J Gastroenterol* 2003; **9**:1904–1908.
16 Wang C, Tang C, Tang L. Inhibition effects of octreotide on the growth of hepatocellular carcinoma *in vitro* and *in vivo*. *Zhonghua Yixue Zazhi* 2001; **81**:1194–1197.
17 Davies N, Cooke TG, Jenkins SA. Therapeutic potential of octreotide in the treatment of liver metastases. *Anticancer Drugs* 1996; **7**:23–31.
18 Buscail L, Delesque N, Esteve JP, Saint-Laurent N, Prats H, Clerc P, *et al.* Stimulation of tyrosine phosphatase and inhibition of cell proliferation by somatostatin analogues: mediation by human somatostatin receptor subtypes SSTR1 and SSTR2. *Proc Natl Acad Sci USA* 1994; **91**: 2315–2319.
19 Hofland LJ, van Koetsveld PM, Waaijers M, Zuyderwijk J, Lamberts SW. Relative potencies of the somatostatin analogs octreotide BIM-23014, and RC-160 on the inhibition of hormone release by cultured human endocrine tumour cells and normal rat anterior pituitary cells. *Endocrinology* 1994; **136**:301–306.
20 Douziech N, Calvo E, Coulombe Z, Muradia G, Bastien J, Aubin RA, *et al.* Inhibitory and stimulatory effects of somatostatin on two human pancreatic cancer cell lines: a primary role for tyrosine phosphatase SHP-1. *Endocrinology* 1999; **140**:765–777.
21 Giannetti N, Enjalbert A, Krantic S. Somatostatin analog SMS 201995 inhibits proliferation in human leukemia T-cell line: relevance of the adenylyl cyclase stimulation. *J Cell Biochem* 2000; **78**:666–673.
22 Chen F, O'Dorisio MS, Hermann G, Hayes J, Malarkey WB, O'Dorisio TM. Mechanisms of action of long-acting analogs of somatostatin. *Reg Pept* 1993; **44**:285–295.
23 di Paolo A, Bocci G, Innocenti F, Agen C, Nardini D, Danesi R, *et al.* Inhibitory effect of the somatostatin analogue SMS 201-995 and cytokines on the proliferation of human colon adenocarcinoma cell lines. *Pharmacol Res* 1995; **32**:135–139.

24 Radosevic-Stasic B, Trobonjaca Z, Lucin P, Cuk M, Polic B, Rukavina D. Immunosuppressive and antiproliferative effects of somatostatin analog SMS 201-995. *Int J Neurosci* 1995; **81**:283–297.

25 Trobonjaca Z, Radosevic-Stasic B, Crncevic Z, Rukavina D. Modulatory effects of octreotide on anti-CD3 and dexamethasone-induced apoptosis of murine thymocytes. *Int Immunopharmacol* 2001; **1**:1753–1764.

26 Nicoletti I, Migliorati G, Pagliacci MC, Grignani F, Riccardi C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. *J Immunol Methods* 1991; **139**:271–279.

27 Fearon ER, Vogelstein BA. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**:759–767.

28 Boyer J, McLean EG, Aroori S, Wilson P, McCulla A, Carey PD, *et al.* Characterization of p53 wild-type and null isogenic colorectal cancer cell lines resistant to 5-fluorouracil, oxaliplatin, and irinotecan. *Clin Cancer Res* 2004; **10**:2158–2167.

29 Lee JM, Erlich RB, Bruckner HW, Szrajer L, Ohnuma TA. Somatostatin analogue (SMS 201-995) alters the toxicity of 5-fluorouracil in Swiss mice. *Anticancer Res* 1993; **13**:1453–1456.

30 Chen TB, Huzak M, Macura S, Vuk-Pavlovic S. Somatostatin analogue octreotide modulates metabolism and effects of 5-fluorouracil and 5-fluorouridine in human colon cancer spheroids. *Cancer Lett* 1994; **86**: 41–51.

31 Romani R, Morris DL. SMS 201.995 (Sandostatin) enhances in-vitro effects of 5-fluorouracil in colorectal cancer. *Eur J Surg Oncol* 1995; **21**:27–32.

32 Weckbecker G, Raulf F, Tolcsvai L, Bruns C. Potentiation of the anti-proliferative effects of anti-cancer drugs by octreotide *in vitro* and *in vivo*. *Digestion* 1996; **57**:22–28.

33 Lamberts SW, van Koetsveld P, Hofland LJ. The interrelationship between the anti-mitotic action of the somatostatin analog octreotide and that of cytostatic drugs and suramin. *Int J Cancer* 1991; **48**:938–941.

34 Patel YC. Molecular pharmacology of somatostatin receptor subtypes. *J Endocrinol Invest* 1997; **20**:348–367.

35 Pollak MN, Schally AV. Mechanisms of antineoplastic action of somatostatin analogs. *Proc Soc Exp Biol Med* 1998; **217**:143–152.

36 Kvols LK, Buck M, Moertel CG, Schutt AJ, Rubin J, O'Connell MJ, *et al.* Treatment of metastatic islet cell carcinoma with a somatostatin analogue (SMS 201-995). *Ann Intern Med* 1987; **107**:162–168.

37 Anthony L, Johnson D, Hande K, Shaff M, Winn S, Krozely M, *et al.* Somatostatin analogue phase I trials in neuroendocrine neoplasms. *Acta Oncol* 1993; **32**:217–223.

38 Burch PA, Block M, Schroeder G, Kugler JW, Sargent DJ, Braich TA, *et al.* Phase III evaluation of octreotide versus chemotherapy with 5-fluorouracil or 5-fluorouracil plus leucovorin in advanced exocrine pancreatic cancer: a North Central Cancer Treatment Group study. *Clin Cancer Res* 2000; **6**:3486–3492.

39 Danesi R, Agen C, Benelli U, Paolo AD, Nardini D, Bocci G, *et al.* Inhibition of experimental angiogenesis by the somatostatin analogue octreotide acetate (SMS 201-995). *Clin Cancer Res* 1997; **3**:265–272.

40 Smith JP, Solomon TE. Effects of gastrin, proglumide, and somatostatin on growth of human colon cancer. *Gastroenterology* 1988; **95**:1541–1548.

41 Dy DY, Whitehead RH, Morris DL. SMS 201.995 inhibits *in vitro* and *in vivo* growth of human colon cancer. *Cancer Res* 1992; **52**:917–923.

42 Qin Y, Schally AV, Willems G. Treatment of liver metastases of human colon cancers in nude mice with somatostatin analogue RC-160. *Int J Cancer* 1992; **52**:791–796.

43 Alonso M, Galera MJ, Reyes G, Calabuig R, Vinals A, Rius X. Effects of pentagastrin and of the somatostatin analog (SMS 201-995) on growth of CT26 *in vivo* adenocarcinoma of the colon. *Surg Gynecol Obstet* 1992; **175**:441–444.

44 Pawlikowski M, Kunert-Radek J, Winczyk K. Differential effects of somatostatin analogues on proliferation of murine colonic cancer cells *in vitro*. *Cytobios* 1997; **89**:183–187.

45 Dy DY, Morris DL. Somatostatin inhibits both in-vitro and in-vivo carcinoembryonic antigen secretion by human colon cancer. *Eur J Surg Oncol* 1993; **19**:168–172.

46 Iftikhar SY, Watson SA, Morris DL. The effect of long acting somatostatin analogue SMS 201.995 therapy on tumour kinetic measurements and serum tumour marker concentrations in primary rectal cancer. *Br J Cancer* 1991; **63**:971–974.

47 He SW, Shen KQ, He YJ, Xie B, Zhao YM. Regulatory effect and mechanism of gastrin and its antagonists on colorectal carcinoma. *World J Gastroenterol* 1999; **5**:408–416.

48 Qin Y, Schally AV, Willems G. Somatostatin analogue RC-160 inhibits the growth of transplanted colon cancer in rats. *Int J Cancer* 1991; **47**: 765–770.

49 Lahlou H, Saint-Laurent N, Esteve JP, Eychene A, Pradayrol L, Pyronnet S, *et al.* sst2 Somatostatin receptor inhibits cell proliferation through Ras-, Rap1-, and B-Raf-dependent ERK2 activation. *J Biol Chem* 2003; **278**: 39356–39371.

50 Pages P, Benali N, Saint-Laurent N, Esteve JP, Schally AV, Tkaczuk J, Vaysse N, *et al.* sst2 somatostatin receptor mediates cell cycle arrest and induction of $p27^{Kip1}$. Evidence for the role of SHP-1. *J Biol Chem* 1999; **274**:15186–15193.

51 Cheung NW, Boyages SC. Somatostatin-14 and its analog octreotide exert a cytostatic effect on GH3 rat pituitary tumor cell proliferation via a transient G_0/G_1 cell cycle block. *Endocrinology* 1995; **136**:4174–4181.

52 Ferjoux G, Bousquet C, Cordelier P, Benali N, Lopez F, Rochaix P, Buscail L, *et al.* Signal transduction of somatostatin receptors negatively controlling cell proliferation. *J Physiol* 2000; **94**:205–210.

53 Petak I, Tillman DM, Houghton JA. p53 dependence of Fas induction and acute apoptosis in response to 5-fluorouracil–leucovorin in human colon carcinoma cell lines. *Clin Cancer Res* 2000; **6**:4432–4441.

54 Peters GJ, van Triest B, Backus HH, Kuiper CM, van der Wilt CL, Pinedo HM. Molecular downstream events and induction of thymidylate synthase in mutant and wild-type p53 colon cancer cell lines after treatment with 5-fluorouracil and the thymidylate synthase inhibitor raltitrexed. *Eur J Cancer* 2000; **36**:916–924.

55 Muller M, Wilder S, Bannasch D, Israeli D, Lehlbach K, Li-Weber M, *et al.* p53 activates the *CD95* (*APO-1/Fas*) gene in response to DNA damage by anticancer agents. *J Exp Med* 1998; **188**:2033–2045.

56 Muller M, Strand S, Hug H, Heinemann E-M, Walczak H, Hofmann WJ, *et al.* Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/ligand system and involves activation of wild-type p53. *J Clin Invest* 1997; **99**:403–413.

57 Liu D, Martino G, Thangaraju M, Sharma M, Halwani F, Shen SH, *et al.* Caspase-8-mediated intracellular acidification precedes mitochondrial dysfunction in somatostatin-induced apoptosis. *J Biol Chem* 2000; **275**:9244–9250.

58 Sadji-Ouatas Z, Lasfer M, Julien S, Feldmann G, Reyl-Desmars F. Doxorubicin and octreotide induce a 40 kDa breakdown product of p53 in human hepatoma and tumoral colon cell lines. *Biochem J* 2002; **364**:881–885.

59 AbdAlla EE, Blair GE, Jones RA, Sue-Ling HM, Johnston D. Mechanism of synergy of levamisole and fluorouracil: induction of human leukocyte antigen class I in a colorectal cancer cell line. *J Natl Cancer Inst* 1995; **87**:489–496.