DIFFERENT PRODUCTION OF TGFβ1 BY CISPLATIN–SENSITIVE AND RESISTANT L1210 CELLS TREATED WITH ANTICANCER DRUGS AND LECTINS

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РАЗЛИЧИЯ В ПРОДУКЦИИ ТФРβ1 ЧУВСТВИТЕЛЬНЫМИ И РЕЗИСТЕНТНЫМИ К ЦИСПЛАТИНУ КЛЕТКАМИ L1210 ПРИ ДЕЙСТВИИ ПРОТИВООПУХОЛЕВЫХ ПРЕПАРАТОВ И ЛЕКТИНОВ

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Cisplatin, methotrexate, vincristin, and cytotoxic lectins (*Viscum album* agglutinin 1 and *Ricinus communis* agglutinin) were shown to induce TGFβ1 production in L1210 murine leukemia cells. Different patterns of TGFβ1 production were observed under the effect of the above mentioned cytotoxic agents in cisplatin-sensitive and resistant L1210 cells. As TGFβ1 inhibits proliferation and induces apoptosis in cisplatin-sensitive L1210 cells, it was suggested that this cytokine can mediate effects of cytotoxic agents.
Key words: **transforming growth factor beta, cisplatin, antitumor drugs, cytotoxic lectins, apoptosis.**

Цисплатин, метотрексат, винкристин и цитотоксические лектины (агглютинин 1 из омелы и агглютинин клещевины) индуцировали продукцию ТФРβ1 в клетках L1210. Установлен различный характер продукции ТФРβ1 под влиянием указанных цитотоксических агентов в чувствительных и резистентных к цисплатину клетках линии L1210. Поскольку ТФРβ1 ингибирует пролиферацию и индуцирует апоптоз чувствительных к цисплатину клеток L1210, высказано предположение, что данный цитокин может опосредовать эффекты цитотоксических агентов.
Ключевые слова: **трансформирующий фактор роста типа бета, цисплатин, противоопухолевые препараты, цитотоксические лектины, апоптоз.**

Resistance of malignant cells to anticancer drugs is an important problem in the treatment of human tumors. Although the induction of genes responsible for multi–drug resistance has been demonstrated in many drug–resistant tumor cells [1], this mechanism cannot explain all cases of low effectiveness of chemotherapy [2].

Earlier we [3] found that L1210 murine leukemia sub–lines possessing various sensitivity to cisplatin–induced apoptosis were also differently induced to death by the TGFβ1. Cisplatin–sensitive cells of this line were dying in the presence of TGFβ1, while cisplatin–resistant cells were refractory to TGFβ1 apoptotic action.

Thus, in can be speculated that TGFβ1 mediates (at least partly) the apoptotic action of cisplatin. Taking into account this suggestion we studied the induction of apo–ptosis by cisplatin and other anticancer drugs as well as by cytotoxic lectins in L1210 cells and the correlation of this action with cellular TGFβ1 production. It should be noted that certain lectins demonstrate a distinct antitumor activity and can be used as an alternative chemotherapy in tumor treatment [4]. We found that highly toxic lectins (*Viscum album* agglutinin 1 (VAA–1) and ricinus agglutinin (RCA–120)) induced TGFβ1 production in studied cells, while the relatively nontoxic Con A did not possess such an activity. All studied drugs and lectins, but Con A, induced TGFβ1 production in cisplatin–resistant L1210 cells. However, this cytokine could not affect these cells due to their resistance to TGFβ1 action.

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Abbreviations used: Con A — concanavalin A; DMEM — Dulbecco's modified Eagle's medium; FCS — fetal calf serum; RCA–120 — ricinus agglutinin; TGFβ — transforming growth factor beta; VAA–1 — *Viscum album* agglutinin 1.

MATERIALS AND METHODS

Cells and their culture. Murine leukemia cells of L1210 line were obtained from the collection of R.E. Ka–vetsky Institute of Experimental Pathology, Oncology and Radiobiology, (Kyiv, Ukraine) and cultured in DMEM (Sigma, USA) supplemented with 10% heat–inactivated FCS (Sangva, Lviv, Ukraine) and 50 μg/ml of gentamycine.

Drugs and lectins. Methotrexate was purchased from Ufa Vitamine Plant (Russia), vincristin — from Faulding Pharmaceutical Co. (USA). Lectins were iso–lated and purified in our laboratory as described [5].

Determination of cell number and viability. In–fluence of anticancer drugs and lectins upon cell growth and viability was studied in 24–well plastic plates. Cells

were cultured for 24 h in FCS–free medium and then 10% FCS and studied drugs or lectins were added for 48 h. At the end of the incubation total cell number and the proportion of dead cells were determined using trypan blue exclusion method.

DNA fragmentation test. DNA preparation and electrophoresis were performed as described [6]. Aliquotes of $5 \cdot 10^6$ cells were pelleted and then resuspended in 50 μl lysis buffer containing 50 mM Tris–HCl (pH 7.5), 20 mM EDTA (Serva, Germany), 1% NP–40. SDS (Serva, USA, final concentration 1%) and RNAse A (Sigma, USA, final concentration 1 mg/ml) were added to each sample, which was they were incubated for 1 h at 37°C. Then proteinase K (Sigma, USA, final concentration 1 mg/ml) was added and samples were incubated for 1 h at 37° C. DNA was pelleted overnight at –20°C after adding 10 M ammonia acetate (1/2 of sample volume) and 2 volumes of ice–cold isopropanol. Samples were centrifuged for 30 min at 10,000 g, pellets were air–dried, dissolved in TE–buffer (10 μl/10^6 cells) and loaded into the wells of 1% agarose gel. Electrophoresis was carried out in TAE–buffer (pH 8.0) in the presence of ethydium bromide and the gel was examined in transilluminator (LKB, Sweden) under UV light and photographed.

ELISA testing of TGFβ1 production. R&D Systems, Inc. (Minneapolis, USA) KIT was used for the study of TGFβ1 presence in the conditioned medium which was collected after the cells were subjected to the action of anticancer drugs or cytotoxic lectins. KIT protocol was strictly followed during testing.

Statistical analysis. Each experiment was performed in triplicate and repeated three times. Significance of the difference in a typical experiment was assessed by Student's *t*–test. The level of significance was set at 0.05.

RESULTS

As one can see in Fig. 1, *a, b* and Fig, 2, *a, b,* cisplatin–resistant L1210 cells were also more refractory to growth inhibiting and killing actions of other anticancer

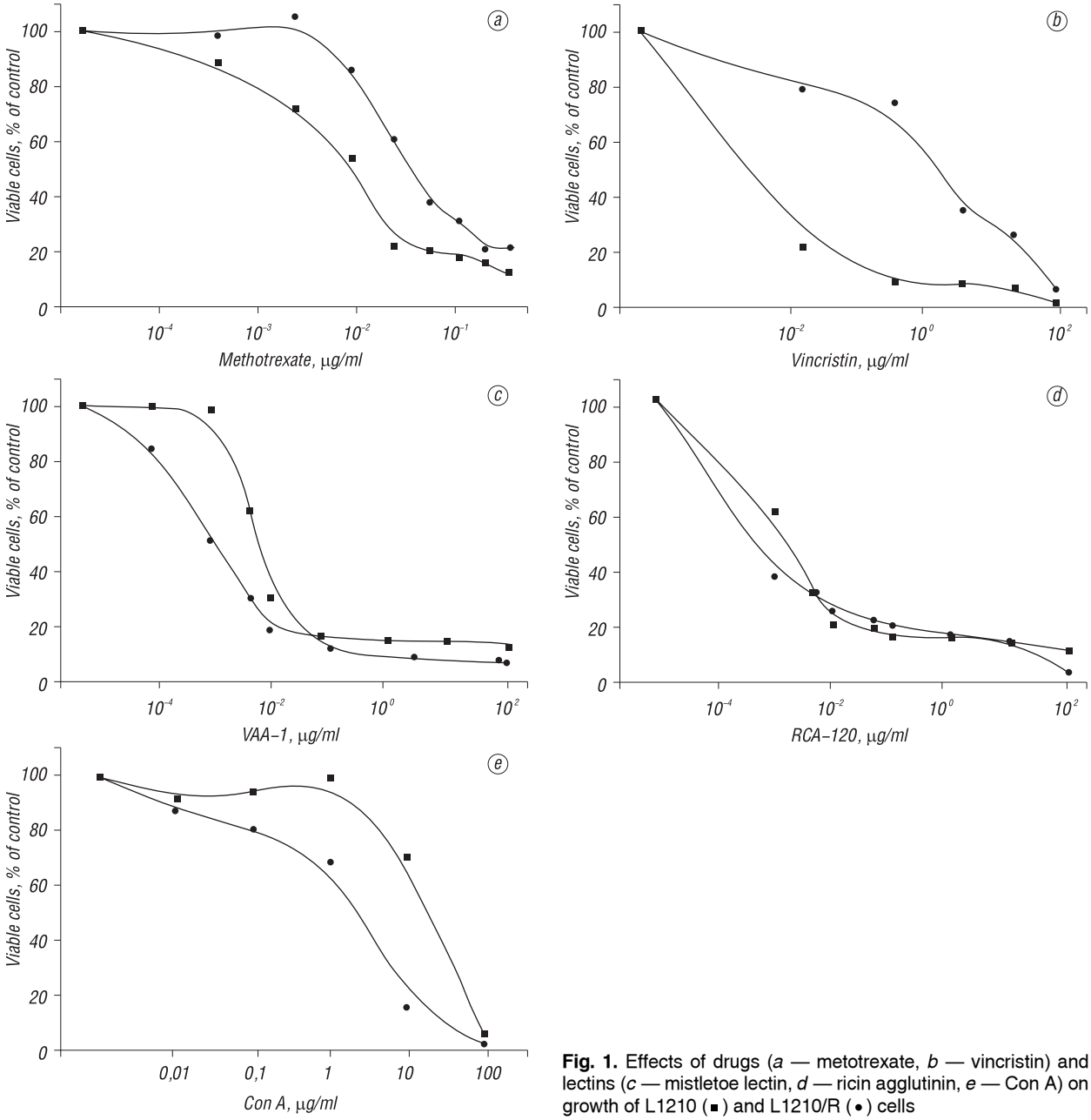

Fig. 1. Effects of drugs (*a* — metotrexate, *b* — vincristin) and lectins (*c* — mistletoe lectin, *d* — ricin agglutinin, *e* — Con A) on growth of L1210 (■) and L1210/R (●) cells

drugs such as methotrexate and vincristin. This difference was not pronounced in the case of cytotoxic lectins (mistletoe lectin (VAA–1) and ricin agglutinin (RCA–120)) action (Fig. 1, *c, d* and Fig, 2, *c, d*). Con A used in concentration up to 1 μg/ml has been shown to be nontoxic for L1210 cells, while in higher concentrations (10–100 μg/ml) it strongly affected these cells (Fig. 1, *e* and Fig. 2, *e*).

Data regarding cell killing activity of anticancer drugs and cytotoxic lectins were in accordance with the ability of these agents to evoke DNA fragmentation (Fig. 3). Methotrexate induced DNA fragmentation in cisplatin–sensitive but not in cisplatin–resistant L1210 cells. Similar picture was observed when these cells were treated with vincristine.

Cytotoxic lectins (VAA–1 and RCA–120) were shown to induce DNA fragmentation in both cisplatin–sensitive and –resistant L1210 cells (see Fig. 3). The effect of Con A strongly depended on its concentration and well–expressed DNA fragmentation was induced only by higher Con A concentrations (25 μg/ml).

Earlier we [3] found that TGFβ1 is an apoptosis–inducing cytokine for L1210 cells, while cisplatin–resistant cells of this line were resistant to TGFβ1 action. Thus, it was reasonable to compare different anticancer drugs and lectins in their ability to induce TGFβ1 production in the studied cells. We found that cisplatin and cytotoxic lectins (but not Con A, 1 μg/ml) induced TGFβ1 production by cisplatin–sensitive L1210 cells (Fig. 4). Methotrexate and vincristin did not possess such ability.

It is worthy to note that TGFβ1 production by cisplatin–resistant L1210 cells was higher than by cisplatin–sensitive cells of this line. Cisplatin could not induce TGFβ1 production by cisplatin–resistant cells, while the methotrexate, vincristin and RCA–120 evoked strong elevation of the production of this cytokine. VAA–1 effect on TGFβ1 production by cisplatin–resistant cells was not so prominent.

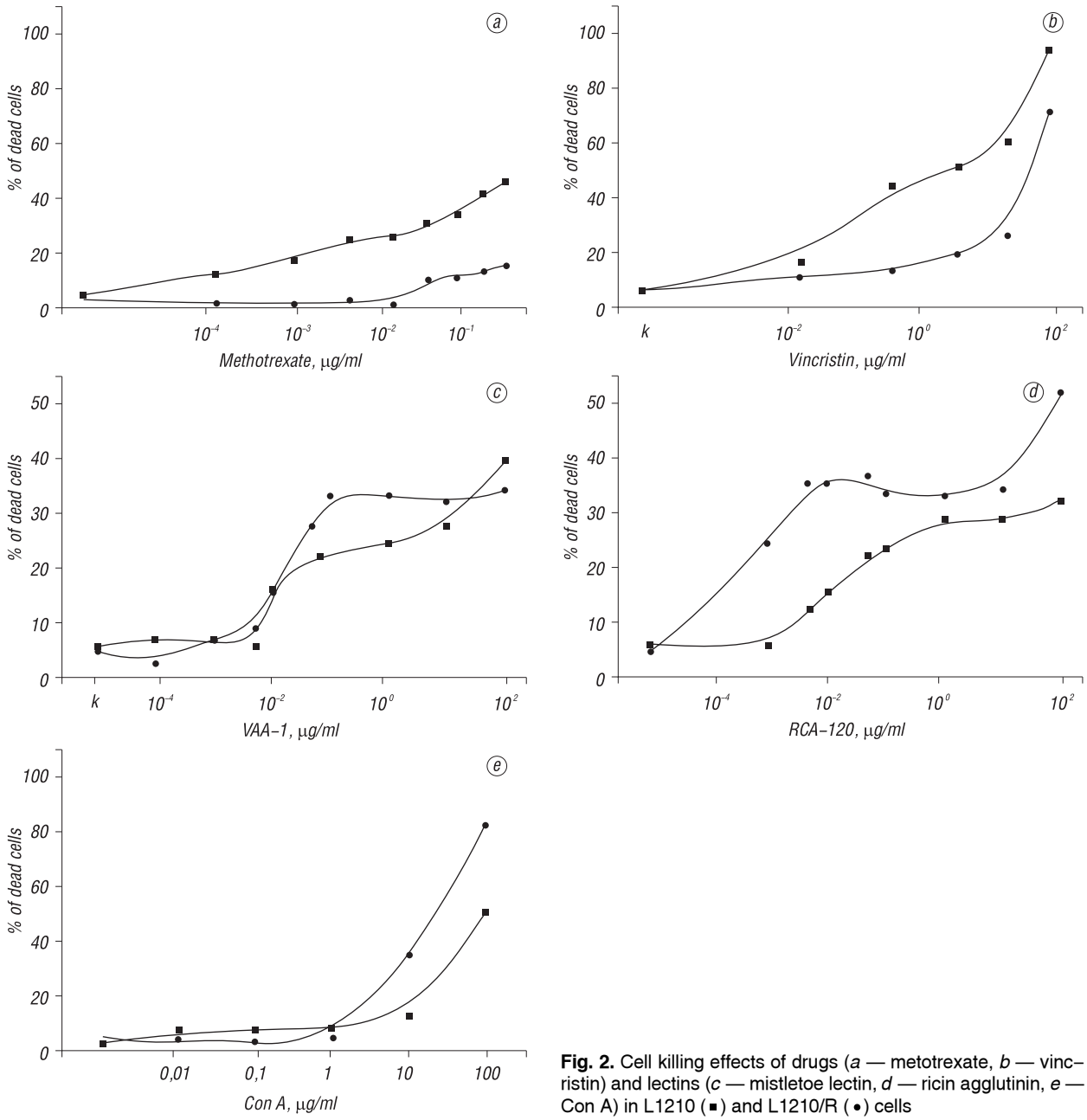

Fig. 2. Cell killing effects of drugs (*a* — metotrexate, *b* — vincristin) and lectins (*c* — mistletoe lectin, *d* — ricin agglutinin, *e* — Con A) in L1210 (■) and L1210/R (●) cells

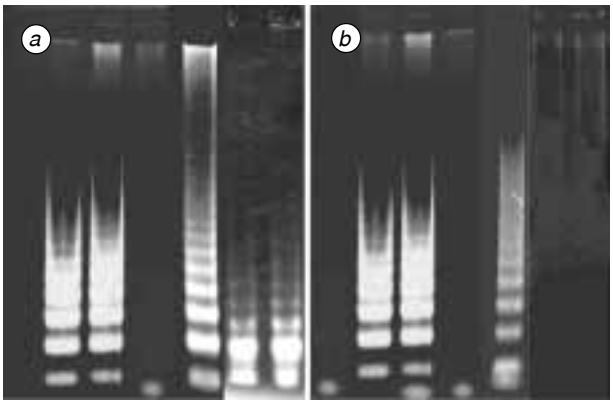

Fig. 3. DNA fragmentation in L1210 (*a)* and L1210/R (*b*) cells: *1* — untreated; *2* – VAA–1 (1 ng/ml); *3* — RCA–120 (1 ng/ml); *4* — Con A (1 μg/ml); *5* — Con A (25 μg/ml); *6* — methotrexate (15 ng/ml); *7* — vincristin (10 ng/ml)

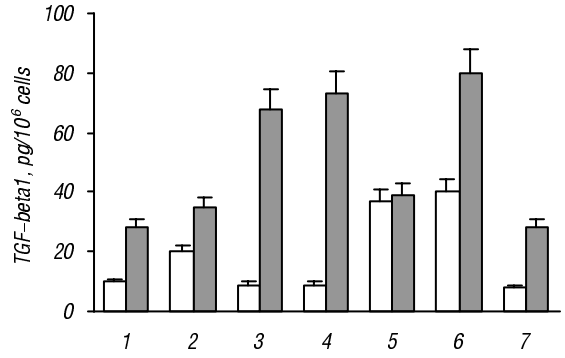

Fig. 4. Effect of different cytotoxic agents on TGFβ1 production by L1210 (□) and L1210/12 cells (■) differing in their sensitivity to cisplatin action: *1* — cells cultured in the medium without cytotoxic agents, *2* — cisplatin (0.1 μg/ml for L1210 and 5 μg/ml for L1210/R); *3* — methotrexate (15 ng/ml); *4* — vincristin (1 ng/ml); *5* — VAA–1 (0.1 ng/ml); *6* — RCA–120 (0.5 ng/ml); *7* — Con A (1 μg/ml)

DISCUSSION

TGFβ1 is a widely spread and very polyfunctional [7, 8] cytokine. It was found that TGFβ1 can induce apoptosis in some cells including malignant ones [9–11]. It is known that malignant cell transformation in some cases is accompanied by specific changes in cell regulation [12]. We have detected that cisplatin–resistant L1210 cells were also refractory to TGFβ1 growth–inhibiting and apoptosis–inducing action [3].

Thus, it was reasonable to check whether cisplatin–sensitive cells can be induced by cisplatin to TGFβ1 production. To know whether the ability of cytotoxic agents to induce TGFβ1 production is a general phenomenon we also studied in parallel experiments the ability of other anticancer drugs and cytotoxic lectins to induce TGFβ1 secretion by L1210 cells.

The anticancer activity of the cytotoxic lectins from castor beans and mistletoe has been known for the relatively long time [13, 14]. The molecules of these lectins consist of two polypeptide chains — A and B. It is believed that the toxic action of these lectins is caused through the inhibition of protein synthesis in ribosomes by the chain A of the lectin molecule, while the chain B is responsible for the interaction with specific receptors on cell surface [14, 15]. Recently, mistletoe agglutinin, especially its isoform I, was introduced as an anticancer drug [16, 17]. The study of the mechanisms of its biological action revealed that besides the protein synthesis inhibition, it possessed several other features, e.g. immunomodulating action, stimulation of cytokine production, induction of apoptosis [18, 19].

The results presented in this paper allow one to suggest that the effect of different cytotoxic agents (anticancer drugs and cytotoxic lectins) on L1210 cells at least partly can be mediated by TGFβ1. This mechanism seems to work well for cisplatin–sensitive L1210 cells in the case of their treatment by cisplatin and cytotoxic lectins (mistletoe lectin and ricin agglutinin). All these agents induced both TGFβ1 production and cell apoptosis. However, it was found that methotrexate and vincristin induced TGFβ1 production in cisplatin–resistant L1210 cells, which are refractory to TGFβ1 growth inhibiting and apoptotic action. Thus, the ability of different cytotoxic agents to induce TGFβ1 production in target cells may not correlate with the apoptotic action of this cytokine, and this happens in the case of target cell resistance to such action of TGFβ1.

The hypothesis about the role of TGFβ1 in mediating action of different cytotoxic agents might be confirmed by the use of specific anti–TGFβ1 antibodies during the cytotoxic agent action. However, there are also indirect data which agree with this hypothesis. It was found in this study that while the cytotoxic lectins induced TGFβ1 production by the tested tumor cells, the relatively nontoxic lectin Con A did not change the production of this cytokine.

It is important to note that in most cases TGFβ1 production by cisplatin–resistant L1210 cells was higher than by cisplatin–sensitive cells of this line. The explanation for this could be that cisplatin–resistant cells were also refractory to TGFβ1 growth–inhibiting and apoptotic action and, thus, TGFβ1 is not harmful for cisplatin–resistant cells.

The results of our study also allow one to suggest that the resistance of L1210 leukemia cells to cisplatin action was due to the impairment in TGFβ1 regulatory pathway (TGFβ1 specific receptors, Smad proteins participating in TGFβ1 signal transduction). The defective expression of TGFβ1 receptor system was detected in colon and gastric carcinomas [20, 21], head and neck squamous carcinomas [22] and of Smad4 protein — in 90% of pancreatic carcinomas [23]. Thus, the restoration of TGFβ1 regulatory system could make the tumors more susceptible to both TGFβ1 and anticancer drugs.

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