Role of Epidermal Growth Factor Gene in the Development of Pancreatic Cancer and Efficiency of Inhibitors of This Gene in the Treatment of Pancreatic Carcinoma

G. F. Muslimov

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The expression of epidermal growth factor receptors in normal and tumor cells of the pancreas, the type and incidence of EGFR gene polymorphism were studied. EGFR gene expression in pancreatic adenocarcinoma cells significantly surpassed that in normal pancreatic cells. On the other hand, AA genome and A allele polymorphism in the EGF gene nucleotide pair G-A 61 is a significant risk factor for pancreatic cancer. The effect of AG-1478 preparation (a new-generation inhibitor of EGFR) on apoptosis and cell proliferation in pancreatic cancer was evaluated. This preparation is not inferior to 5FU by its apoptotic effect and significantly reduces cell proliferation, its antiproliferative effect being 1.5 times higher than that of 5FU.

Key Words: *pancreatic cancer; EGFR; gene polymorphism; apoptosis; cell proliferation*

The mean life span of patients with pancreatic cancer (PC) is less than 6 months, 12-month survival is noted in 10% and 5-year survival in just 3-5% patients [1,4,7,10]. By the moment of PC diagnosis only 10-15% patients are candidates for surgical treatment, after which the mean life span is 10-18 months, and only 17-24% patients survive during 5 years [14].

Recent studies led to significant improvement of PC diagnosis, adjuvant and nonadjuvant therapy; the results of studies of PC molecular oncogenesis were particularly interesting [3,10,11,14,15].

Molecular biological studies showed that isoforms of developmental factors and their receptors play an important role in PC formation [6,9]. Epidermal growth factor (EGF) and its receptors (EGFR) attract special interest. The signal activated by EGFR, penetrating into cell nucleus, accelerates protooncogene phosphorylation, and it is therefore assumed that a serious prognosis of PC is also due to high expression of EGFR and its gene polymorphism.

Clinical efficiency of therapy with EGFR monoclonal inhibitors after radical surgery for PC and their combinations was demonstrated [12].

Inhibition of EGFR is considered to be one of the targets of adjuvant and nonadjuvant therapy for PC [6,9,14,15]. Other objects of modern studies are molecular inhibitors of tyrosine kinase activities. However, the efficiency of EGFR inhibitors in PC is not documented by clinical or experimental studies.

We evaluated the level of EGFR expression, analyzed the incidence of EGF gene polymorphism in PC patients, and experimentally studied the effects of AG-1478 (AG), a new-generation inhibitor of EGFR, on apoptosis and cell proliferation in PC.

M. Topchubashov Research Center of Surgery, Baku, Azerbaijan. ***Address for correspondence:*** dr_gurban@yahoo.com. G. F. Muslimov

MATERIALS AND METHODS

The study was carried out at laboratory of experimental studies (head: Prof. G. Allhaer), Mannheim Surgical Clinic, Heidelberg University (Germany).

Immunohistochemical studies for evaluating EGFR expression were carried out on tumor and intact tissue biopsy specimens from 125 patients with histopathologically confirmed PC.

Mononucleotide gene polymorphism for EGF-ALU1 was studied after cleavage of PCR products with specific endonucleases by reaction-restriction fragment length polymorphism analysis (PCR-RFLP).

Experimental studies were carried out on PC strain PaTu 8902.

Apoptosis in PC was evaluated by Vybrant^{R} Apoptosis Assay Kit 2, proliferation by ELISA reading system (Model 550, Bio-Rad) and Beckman Coulter Analysator.

The effect of AG (new-generation inhibitor of EGFR) on apoptosis and cell proliferation was studied in 4 groups. In group 1 (control), tumor cells were incubated without treatment, in group 2 with 5FU (25 μM), in group 3 with AG (50 μM), and in group 4 with 5FU (25 μM) and AG (50 μM).

Incubation was carried out in a CellTiter 96 Aqueous One Solution device; 20 ml reagent was put into the wells after the drug. Incubation was carried out for 2-4 h. Cell proliferation was evaluated using the ELISA reading system at 490 nm; the counts of apoptotic, living, and dead cells were evaluated by fluorocytometry (FACS).

RESULTS

Normally, EGFR expression in all tissues, including the pancreatic tissue, is low. Immunohistochemical analysis of EGFR expression was carried out (Fig. 1). The expression of transmembrane local EGFR in tumor cells was significantly higher than in normal pancreatic cells (Fig. 1, *d*).

The next step was analysis of genome and allele polymorphism types and their incidence in PC patients by the PCR-RFLP method (Fig. 2). The

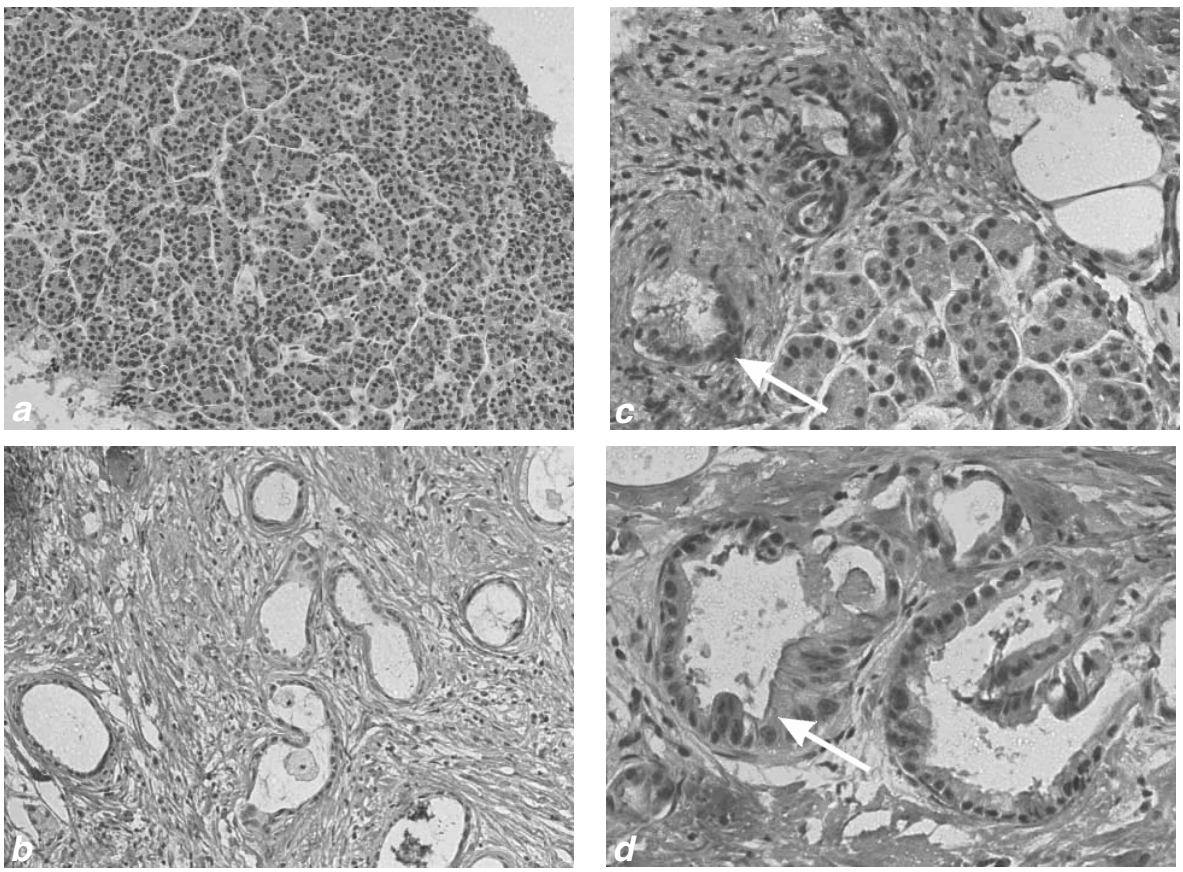

Fig. 1. Comparative immunohistochemical analysis of EGFR expression in pancreatic adenocarcinoma. *a*) unstained (negative) normal pancreatic tissue; *b*) unstained tumorous pancreatic tissue; *c*) normal pancreatic tissue stained by EGFR monoclonal antibodies; *d*) tumor tissue stained by EGFR monoclonal antibodies. Arrow shows expression of transmembrane local EGFR.

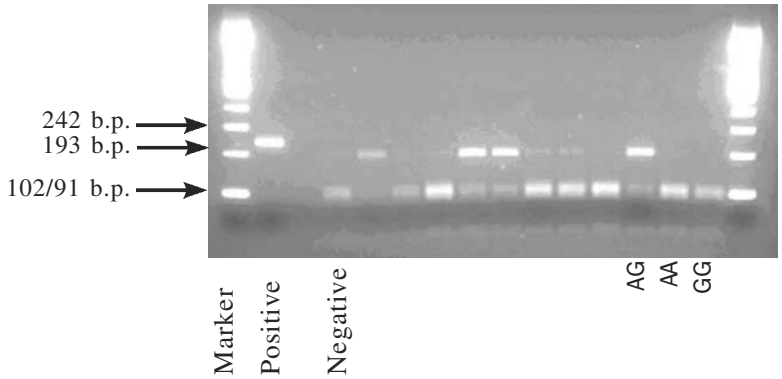

Fig. 2. EGF gene isoforms (PCR-RFLP method).

incidence of various types of genome and allele polymorphism is presented in Figs. 3 and 4.

No appreciable differences in the EGF nucleotide pair 61 G-A were detected for AG and GG genomic polymorphisms and G allele polymorphism. On the other hand, the groups differed significantly by AA genomic and A allele polymorphisms. The AA genome was detected in 33.6% of PC patients and in only 9.1% controls ($p<0.01$). In healthy volunteers, allele A constituted 32.7% of genotype *vs.* 50.8% in the patients ($p<0.01$).

The time course of cytometry values 24, 48, and 72 h after therapy with different drugs is presented in Figure 5.

One day after treatment apoptosis values in the 5FU group were significantly higher than in groups 3 and 4 (2.2 and 1.4 times, respectively). Hence, at this stage of the study the number of normal and dead cells surpassed that of apoptotic ones.

Two days after treatment apoptosis values in all groups reached the maximum levels, which was in line with published data. The data in groups 3 and 4 were virtually the same and, similarly as during the previous period, differed from the value in group 2. The parameters in groups 2 and 3 did not differ significantly (apoptosis of 53.74 and 44.08% cells, respectively).

After 72 h apoptosis values decreased in all groups. The highest level was observed in group 4 (5FU+AG): the number of apoptotic cells was 1.4 times higher than in group 2 and 1.3 times higher than in group 3. Hence, apoptosis values 72 h after the treatment decreased 1.7 times in group 2 and 1.5 times in group 3 in comparison with the values 48 h after treatment. In group 4 apoptosis value reached 40.28% (increased 1.1 times; $p<0.001$) by this term.

One of informative methods for evaluating the efficiency of adjuvant and nonadjuvant therapy of malignant tumors of the gastrointestinal tract is the study of cell proliferation. Cell proliferation was evaluated 24, 48, and 72 h after the treatment.

The lowest level of proliferation on day 1 after treatment was observed in group 4 (AG+5FU). The efficiency of AG after 24 h was higher than that of 5FU. The time course of cell proliferation after

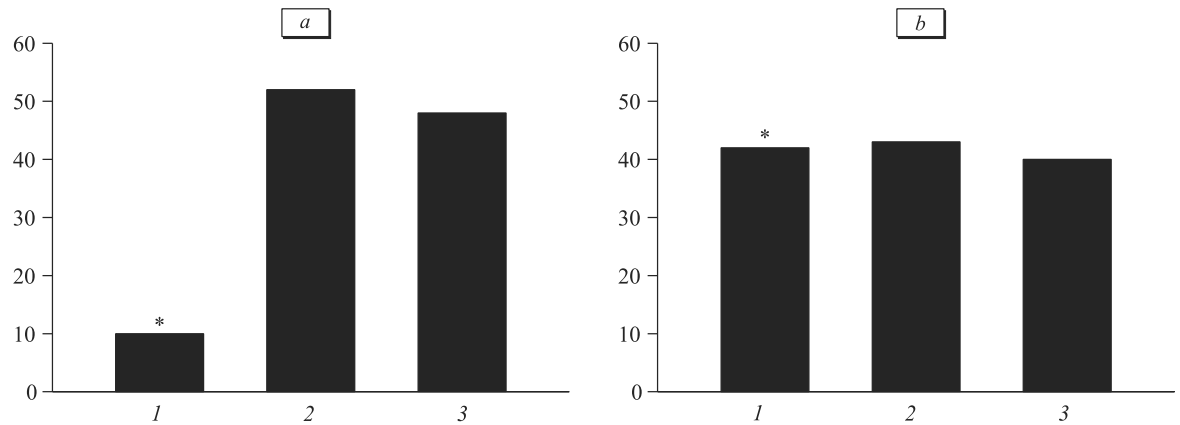

Fig. 3. Incidence of various types of genomic polymorphism in the EGF nucleotide pair 61 G-A in PC patients. *a*) normal subjects; *b*) patients. *1*) AA; *2*) AG; *3*) GG. *$p<0.05$ (χ^2 test).

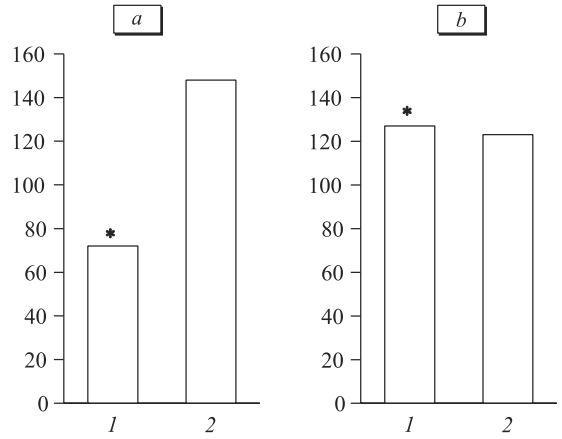

Fig. 4. Incidence of allele polymorphisms in EGF nucleotide pair 61 G-A in PC patients. *a*) normal subjects; *b*) patients. *1*) allele A; *2*) allele G. *$p<0.05$ (χ^2 test).

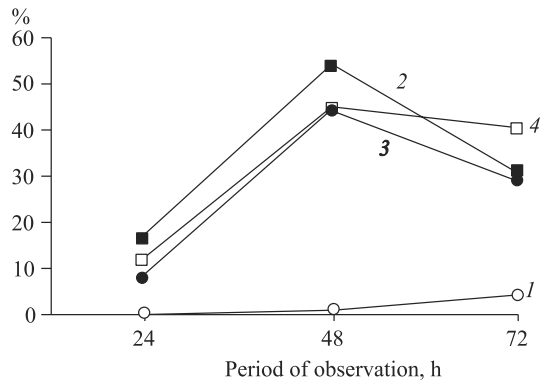

Fig. 5. Time course of apoptosis in different groups. *1*) control; *2*) 5FU; *3*) AG; *4*) 5FU+AG.

48 h indicated higher antiproliferative efficiency of AG (group 3).

On day 2 of treatment (48 h) the highest antiproliferative effect was observed in group 4. At this stage of the study, AG was more effective than 5FU. After 48 h antiproliferative activity increased 5-fold in the AG group and 1.5 times in the 5FU group in comparison with the values on day 1.

As expected, cell proliferation started to decrease in the control group after 72 h, while the efficiency of AG, 5FU, and AG+5FU was still increasing.

We conclude from these results that the expression of EGFR gene in PC tissues is significantly higher than in normal pancreatic tissue. The AA genome and allele A polymorphism in the EGF gene nucleotide pair 61 G-A is a significant risk factor for PC development. The effect of AG preparation on cell apoptosis in PC was not inferior to that of 5FU (maximum apoptotic effect due to AG was 44.08%, that of 5FU 53.74%), while the AG+5FU combination was the most effective. The AG preparation effectively reduced cell proliferation in PC and by antiproliferative effect was 1.5 times more active than 5FU.

Hence, AG, a new-generation inhibitor of EGFR, can be considered as one of the most effective drugs for adjuvant and nonadjuvant therapy of PC.

REFERENCES

1. A. Gustin, L. Pederson, R. Miller, *et al.*, *World J. Surg.*, **26**, No. 7, 854-860 (2002).
2. C. L. Arteaga, *Semin. Oncol.*, **29**, No. 5, Suppl. 14, 3-9 (2002).
3. N. Bardessi and A. R. DePinho, *Cancer*, No. 2, 897-909 (2002).
4. *Cancer Facts and Figures*, Atlanta (2001), pp. 5-61.
5. P. Ghaneh, A. Kawesha, J. D. Evans, and J. P. Neoptolemos, *J. Hepatobiliary Pancreat. Surg.*, Vol. 9, No. 1, 1-11 (2002).
6. P. Ghaneh, J. Seavin, R. Satton, *et al.*, *World J. Gastroenterol.*, **7**, No. 4, 482-489 (2001).
7. R. T. Greenlee, M. B. Hill-Harmon, T. Murray, and M. Thun, *Cancer J. Clin.*, **51**, No. 1, 15-36 (2001).
8. J. Mendelsohn, *Endocr. Rel. Cancer*, **8**, 3-9 (2001).
9. M. G. Raraty, C. J. Magee, P. Ghaneh, and J. P. Neoptolemos, *Acta Oncol.*, **41**, Nos. 7-8, 582-595 (2002).
10. M. Mimeault, R. E. Brand, A. A. Sasson, and S. K. Batra, *Pancreas*, **31**, 301-316 (2005).
11. E. Missiaglia, E. Blaveri, B. Terris, *et al.*, *Int. J. Cancer*, **112**, No. 1, 100-112 (2004).
12. J. P. Neoptolemos, D. Cunningham, H. Friess, *et al.*, *Ann. Oncol.*, **14**, No. 5, 675-692 (2003).
13. C. L. Vogel, M. A. Cobleigh, D. Tripathy, *et al.*, *J. Clin. Oncol.*, **20**, No. 3, 719-726 (2002).
14. S. Westphal and H. Kalthoff, *Mol. Cancer*, **2**, No. 6, 1-14 (2003).
15. M. Yamada, T. Ikeuchi, and H. Hatanaka, *Prog. Neurobiol.*, **51**, No. 1, 19-37 (1997).