Ribonucleases (RNases) are an exclusively various group of enzymes, capable to degrade various kinds of RNA. Each of principal species of RNA (ribosomal, transport and messenger) has quite certain duration of existence supervised by a certain class of RNA-splitting enzymes and corresponding inhibitors.

Earlier it was considered that RNases carry out only one certain function- to degrade overaged molecules of RNA. Further, however, it was found that functions of these enzymes are much wider and fall outside the limits of simple destruction of RNA molecules. RNases carry out the basic function in processes of splicing [1] and processing [2] of polynucleotides. In cases of alternative splicing RNases can considerably change the structure of synthesized protein molecules encoded by a certain gene, and therefore can be synthesized not one, but various protein products possessing the different properties. Additional influence on the destiny of various polynucleotides can render ribozymes [3–5] and numerous of RNase inhibitors, ions of metals, and also local values of pH inside of various cell compartments. Notorious opinion is that in rapidly proliferating cells activity of RNases are lowered, that is dictated by requirement of significant amounts of RNA for protein synthesis. Really, in the cells of many tumours activity of RNases is lowered [6–8]. At the same time there are exceptions. At malignant neoplasms of pancreas the level of RNases in tumour tissue and in a blood plasma is raised [9–11]. Moderately elevated values of serum RNase activity was found in patients with 6 different neoplasms, but strikingly abnormal elevations occur in serum of patients with pancreatic cancer [9]. The conclusion was made that abnormal elevation of serum RNase activity serves as reliable biochemical marker of carcinoma of the pancreas in the presence of the normal renal function [9]. In later research by Doran et al. [11] serum RNase activity has been measured in 61 patients with a variety of inflammatory and malignant conditions. Serum RNase activity is elevated in patients with pancreatic carcinoma, but it is not restricted to this condition. Activity was significantly elevated in cases of disturbed liver function [11].

In blood plasma and urine of patients with a chronic myeloid leukaemias RNase activity raises significantly [12]. High RNase activity of eosinophil granules allows to assume the important role of this enzyme at an eosinophilia and its participation in allergic reactions [13, 14]. Ambiguous were finding the changes of RNases activity at a prostate cancer [15–18]. Recently the interferon antiviral pathways and prostate cancer genetics have converged on a specific endoRNase. Studies from laboratories the USA, Finland and Israel suggest that mutations in RNAseL (interferon-induced latent RNase, RNaseL) gene predispose men to incidence of prostate cancer. In some cases such mutations reflect more aggressive disease and/or decreased age of onset compared with non-RNASEL linked cases [15]. Being based on these data certain authors suggest a relationship between innate immunity and tumor suppression. The RNASEL gene encodes a single-stranded specific endoRNase involved in the antiviral actions of the interferons. Enzyme is activated after binding to unusual 2'-5' oligoadenilates (2-5A). Stable phosphorothioate analogues of 2'-5A synthesized chemically induced RNaseL activity and caused apoptosis in cultures of metastatic human prostate cancer cell lines. The deficiency in RNaseL activity was correlated with a reduction its ability to dimerize into catalytically active form [16]. Because Afro-Carribeans are at high risk of developing prostate cancer, Shea et al. [17] re-sequenced the positional candidate gene RNASEL in 48 prostate cancer cases and genotyped the previously reported polymorphisms in 230 prostate cancer cases and 458 controls. Results
obtained suggest that variation in the putative cancer susceptibility gene *RNASEL* or its inhibitor does not contribute significantly to prostate cancer risk in this population [17]. Also Larson et al. [18] found that prostate cancer in patients with R4 620 allelic mutation in *HPC1/RNASEL* gene is not associated with more aggressive clinical or pathological features in specimens investigated.

Angiogenin, a protein capable of promoting angiogenesis and possessing RNase activity was found to be markedly elevated among a sub-group of patients with progressive melanoma. High angiogenin levels were significantly associated with poor treatment response with chemoimmunotherapy. Treatment-related survival (TRS) was shorter (10 months) in patients with above-median values than in those with below-median levels (19 months). These data suggest that serum RNase angiogenin might be of predictive value in the evaluation of treatment response for patients with melanoma [19].

Some authors believe that RNases serve as cytotoxic agents during host defense and provide physiological cell death pathways. In bacteria, plants and mammals RNases can bind target cells, degrade RNA and kill the cells. Such events occur in the interstrain competition in bacteria, in the death of incompatible pollen in the higher plants and play role in the antiparasitic and anticancer activity of eosinophils in man [14, 20]. The eosinophil-derived neurotoxin (RNase) has been suggested to be the putative anti-Kaposi’s sarcoma cells compound [14]. RNase H1 takes place in generation of mitochondrial DNA. Cerritelli et al. [21] found that RNaseH−/− mice resulted in significant decrease in mitochondrial DNA content leading to apoptotic cell death.

Immunosuppressive cytokine interleukin-10 possesses DNA and RNA hydrolyzing activity due to structural homology with endogenous differentiation factor for the HL-60 line of human promyelocyte leukemia cells. The human recombinant interleukin-10 was shown to cleave all forms of plasmide DNA and plays significant role in apoptosis induction in monocyte cells [22].

Ability of RNases to degrade various kinds of RNAs and block protein synthesis began an occasion to attempts to use RNases as the antitumor drugs. Pancreatic RNase A can exhibit cytotoxic activity. This toxicity relies on cellular internalization of the enzyme. Fuchs and co-workers [24] found that replacing Glu49 and Asp53 with arginine does not affect catalytic activity or affinity for the cytosolic RNase inhibitor. This “arginine graft” does however, increase toxicity towards human cancer cells. Appending a nonarginine domain to this cationic variant of enzyme results in an additional increasing cytotoxicity, providing one of the most known cytotoxic variants of RNase A [24]. These findings correlate the potency of a RNase with its deliverance of ribonucleolytic activity to the cytosol, and indicate a rationale to enhance cytotoxic efficacy of RNases and other proteins.

Several RNases, including onconase and alphasaracin, are known to be toxic to tumor cells. RNase T1, although its structure is related to that of alpha-saracin, is noncytotoxic because of its inability to internalize into tumor cells. Yuki and others found that when RNase T1 was internalized into human tumor cells via a novel gene transfer reagent, hemagglutinating virus of Japan (HVJ) envelope vector this resulted in cell death. This cytotoxicity was powerfully increased by pretreatment of HVJ envelope vector with protamine sulfate and was stronger than that of onconase, which is in phase III human clinical trials as nonmutagenic cancer chemotherapeutic agent [25]. RNase T1 induced program of apoptotic cell death. Because of this cytotoxicity is not specific to tumor cells, enzyme can not be developed as anticancer drug, but authors believe that other enzymes incorporated in HVJ vector will be a unique anticancer drug if HVJ vector can be targeted to tumor cells.

Ribotoxins are a family of highly specific fungal RNases that inactivate the ribosomes by hydrolysis of a single phosphodiester bond of the 28S rRNA. Enzyme promotes apoptosis of human tumor cells after internalization via endocytosis. This ability is related to its interaction with phospholipid bilayers and shared structured core with nontoxic RNases of the RNase T1 family. Garcia-Ortega et al. [26] found that deletion of the NH2-terminated β-hairpin of the ribotoxin α-saracin produces a nontoxic but active RNase. Results open a possibility of engineering RNases for the preparation of specific cytotoxins.

The antiproliferative action of the guanine-specific RNase secreted by *Bacillus intermedius* (binase) was studied in different chicken and mouse cell lines. The proliferation rate of chicken embryo fibroblasts, either normal or Rous sarcoma virus transformed was significantly reduced by binase treatment. Among mouse fibroblasts V-Ras transformed NIH 3T3 cells were sensitive to binase, whereas the growth of nontransformed, v-src-transformed or v-fms-transformed NIH 3T3 cells was not affected [27]. Considering the insufficient antitumor activity of natural RNases, there have been begun attempts to modify their
structure on purpose to increase their transmembrane permeability, or cytotoxicity concerning tumor cells.

Chemically coupling RNases to new binding moieties or fusing RNase genes to antibody genes results in chimeric molecules with specified cell-type cytotoxicity. This allows the use of human enzymes instead of plant and bacterial toxins, in the construction of immunotoxins. RNases can be engineered to kill cells by cytosolic expression or to kill viruses by packaging into viruses.

Engineering RNases into cell-type-specific cytotoxins may result in a new class of therapeutic agents. De Lorenzo et al. [28] reports the preparation and characterization of fully human antitumor immuno-RNase (IR). Human RNase and fusion protein made up of a human single chain of variable immunoglobulin fragment is directed to the ErbB2 receptor that overexpressed in many carcinomas [28]. Anti-ErbB-2 immuno-RNase retains the enzymatic activity of the wild-type enzyme and specifically binds to ErbB-2 — positive cells with high affinity. Enzyme behaves as an immunoprotoxin. Administered in five doses of 1.5 mg/kg to mice bearing an ErbB-2-positive tumor hErb-hRNase induced a dramatic reduction in tumor volume [28].

Two anti-Erb2 immunoconjugates, called Erb-hRNase (erbicin) and Erb-hc Ab have been prepared by De Lorenzo et al. [29]. It was found that conjugates were selectively cytotoxic on ErbB2-positive cancer cells in vitro and in vivo. In Erb-hRNase, erbicin was linked to the key structural and functional regions of a human IgG [29]. Authors found that antitumor action of Herceptin and that of the novel agents were significantly increased in an additive fashion. An inspection of the mechanism of action of Erb-hRNase or Erb-hcAb combined with Herceptin provided evidence that the antibody combinations engendered an increased downregulation of the ErbB2 receptor, and led to an enhanced apoptotic cell death.

Pouckova et al. [30] show that polymer-conjugated bovine pancreatic and bovine seminal RNases inhibit growth of human tumors in nude mice.

Suzuki et al. [31] mutated two human RNases — pancreatic RNase and eosinophil-derived neurotoxin to incorporate cysteine residues at putative sites of close contact to RNase inhibitor but distant to catalytic sites. Coupling of Cys89 of RNase and Cys87 of eosinophil-derived neurotoxin to proteins at these sites via thioether bond produced enzymatically active conjugates that were resistant to inhibitor. The transferrin-recombinant human RNase thioether conjugate was 5000 fold more toxic to U251 cells than recombinant wild-type hRNase. Transferrin-targeted eosinophil-derived neurotoxin exhibited tumor cell toxicities similar to those of hRNase. So, authors endowed two human RNases sensitive to inhibitor with greater cytotoxicity by increasing their resistance to inhibitor. This strategy shows the possibility to generate a novel set of RNases useful for targeted strategy of cancer therapy [31].

Rutkosky et al. [32] designed variants of RNase A that evade of RNase inhibitor. Three related variants of RNase A were more resistant to cytosolic inhibitor and more toxic to human cancer cells than was amphibian RNase (onconase). Results obtained by researchers demonstrate the utility of the new technology in the examination of protein-protein interfaces and represent a landmark towards the goal of developing chemotherapeutics based on mammalian RNases.

Recently, hydrophilic poly[N-(2-hydroxypropyl)methacryl-amide] (PHPMA) was used for BS-RNase modifications to prevent its degradation in bloodstream or fast elimination [33]. Polymer-conjugated bovine seminal RNase preparation proved to be cytotoxic after intravenous or intraperitoneal application, whereas native BS-RNase was ineffective. RNase unimer was conjugated with two HPMA polymers and their antitumor effects both in vitro and in vivo were compared with those of BS-RNase polymers. Surprisingly, the antitumor effect of RNase A conjugates were also pronounced. The RNase conjugates injected intravenously to mice bearing melanoma tumor caused significant reduction in tumor volume following ten doses of 5 and 1 mg/kg, respectively. Despite the antitumor activity observed in vivo, the in vitro tested cytotoxic activity of RNase A did not differ from that caused by native RNase A, while native BS-RNase (50 μg/ml) totally inhibited DNA synthesis in treated cells. The experiments with 125I-labeled preparations demonstrated concentration-dependent internalization of native BS-RNase by tumor cells within an 1 h, whereas the polymer conjugate (S-BS) was not internalized. On the contrary, the in vivo experiments showed that whereas 40% of S-BS conjugate persisted in blood stream for 24 h after administration, 98% of the native BA-RNase was already eliminated [33].

Interestingly, RNases selectively kill tumor cells via induction of caspases activity and promoting apoptosis [34–37].

Ranpirnase is an amphibian oocyte or early embryo RNase of 105 amino acids in length that is capable of controlling tumor growth by degrading RNA withing cancer cells, resulting in inhibition of protein synthesis and arresting mitosis in G1 phase. It represents the first successful isolation, purification and characterization of the embryonic factor capable controlling cell growth of the early embryonic tissues [38]. This enzyme is a novel class of cancer chemotherapeutic agents, based on homologs and variants of bovine pancreatic RNase. Ranpirnase in combination with doxorubicin is in clinical trials for the treatment of nonresectable malignant mesothelioma and other cancers. The putative mechanism for ranpirnase cytotoxicity involves binding to anionic components of the extracellular membrane, cytosolic internalization and degradation of transfer RNA leading to apoptosis [39].

The maintenance of ribonucleolytic activity in the presence of the cytosolic RNase inhibitor is a key aspect of the cytotoxic activity of ranpirnase.
But the real basis of the specific toxicity against cancer cells is not known. In cell culture studies ONC significantly inhibited tumor growth of A549 human non-small cell lung carcinoma (NSCLC) cells without damaging non-cancerous cells (HLF-1 human lung fibroblasts). Multiple small doses of ONC significantly prolonged tumor growth of A549 tumors, with increased apoptosis in vivo from 0.5 ± 0.3 to 70 ± 1.1%. Interestingly, multiple small doses of ONC were more effective than a single large dose for the tumor growth inhibition with minimal side effects [38]. Alfacell Corporation is conducting phase III of registration trials of ranpirnase plus doxorubicin versus doxorubicin alone in more than 360 patients with nonresectable malignant mesothelioma, and will assess survival as the primary endpoint. The targeted treatment group in this trial represents 90% of malignant mesothelioma patients at the time of diagnosis. The trial is being conducted in the US, Canada, Poland, Italy, Germany, Australia, New Zealand, Russia, Romania, Mexico, and Brasil [38–41]. Cytotoxic RNases such as ranpirnase, represent a novel mechanism-based approach to anticancer therapy. Based on phase I study data the maximum tolerated dose (MTD) was found 960 microg/m² with the dose limiting toxicity (DLT) characterized by proteinuria with or without azotemia, peripheral edema and fatigue. Ranpirnase did not induce myelosuppression, mucositis, alopecia, cardiotoxicity, coagulopathy, hepatotoxicity or adverse metabolic effects. Phase II tumor-specific trials investigated the activity of ranpirnase in malignant mesothelioma, breast cancer, non-small cell lung cancer, and renal cell cancer. A phase III randomized study in malignant mesothelioma patients compares the combination of ranpirnase plus doxorubicin to doxorubicin monotherapy [40, 42].

Onconase (ONC), isolated from amphibian oocytes was used to study its effect on the radiation response in A549 human NSCLC in vitro and in vivo [41]. In cell culture studies authors found that ONC increased the radiation response by ONC-induced inhibition of O₂ consumption. The occurrence of apoptosis was increased by ONC and was dependent on doasages and time exposure (measured by a TUNNEL assay). Moreover, ONC inhibited sublethal damage repair in a split-dose experiment. In animal studies ONC significantly increased the radiation-induced tumor growth delay of A549 tumors in vivo. Authors concluded that the ONC-induced enhancement in tumor oxygenation was mainly due to the reduction in QO₂ rather than an increase in tumor blood flow [40]. Administration of 1.67 μM ONC into cultures of HLF60 cells led to appearance of cells that had features characteristic of apoptosis. Studies indicated that ONC induces apoptosis of the target cells most likely along the mitochondrial pathway involving caspase-9 as the initiator caspase [39]. Interestingly, that during apoptosis caspases and Ser-proteases may transactivate each other [37].

The generation of micro RNAs is dependent on the RNase III enzyme DICER, the levels of which vary in different normal cells and in disease states.

Type I interferons repress DICER protein in contrast to IFN-gamma, which induces DICER [43]. In this connection ribonomic profiling and strategic merging genomic technologies was proposed for the induction or interruption of cellular growth in cancer [44].

The discovery that RNA can act as hydrolytic enzyme apart from carrying genetic information has given a new direction to the gene therapy. Ribozymes can be used to down-regulate by RNA cleavage or repair by RNA transsplicing unwanted gene expression involved in cancer [45, 46]. Powers et al. [4] reduced tyrosine kinase of anaplastic glioma (ALK) by ribozyme targeting and demonstrated that this prevents pleiotrophin-stimulated phosphorylation of the anti-apoptotic protein Akt. This depletion of ALK reduced tumor growth of the xenografts in athymic nude mice and prolonged survival of the animals because of increasing of apoptosis in the tumors.

There is, however, a number of certificates in favor of that some RNases can stimulate tumor growth. Some of them are produced by tumor cells. There is a number of certificates that suppression of activity of certain intracellular RNases can have inhibiting effect on a cellular proliferation and tumor growth.

Angiogenin (ANG), a 14.4 kDa monomeric basic protein was originally identified as tumor-derived angiogenesis factor [47]. However, further it was shown that angiogenin possesses RNase activity. Monoclonal antibodies and an antisense oligonucleotides directed against angiogenin inhibited growth of human colon, prostate, breast, lung and fibroblast tumors in athymic mice [48–50]. ANG mRNA and protein was elevated in colorectal [51, 52], gastric [51], pancreatic [53], breast [54, 55], prostate cancers [56], and melanoma [57], comparing with corresponding normal tissues. In some cases high level of ANG was shown to correlate with cancer progression or poor prognosis [52, 53, 58]. ANG was significantly increased in serum of patients with gastric [59], pancreatic [53], ovarian [60], renal cancer [61], and melanoma [62]. An attractive strategy for developing anti-angiogenic drugs is a ribonucleolytic activity of ANG. Enzymic activity is essential for angiogenesis [63–65]. ANG is a member of bovine pancreatic RNase A superfamily and possess 33% sequence identity to pancreatic enzyme [66]. Kao et al. [67] have chosen from 18310 compounds [8-amino-5-(41-hydroxybiphenyl-4-ylazo) naphthalene-2-sulfonate] (NCI 65828) that possessed the RNase inhibiting activity. Local treatment with modest doses of NCI 65828 significantly delayed the formation of tumors from two distinct human cancer cells in athymic mice [67, 68]. At the same time the compound 65828 at concentrations up to 100 μM did not inhibit culture growth of PC-3, HT-29, or any of 57 other tumor cell lines tested. Thus, antitumor activity of this compound does not depend on the direct effect...
on tumor cells but are consistent with inhibition of angiogenesis.

Polakowski et al. [69] found that recombinant RNase inhibitor could inhibit angiogenesis and reduce tumor growth in adult mice. Cell penetration into a polyvinyl alcohol sponge was reduced to 29% of control (PBS or heat inactivated RNase inhibitor). Inhibitor caused significant reduction in mammary tumor growth that autors devoted to angiogenesis inhibition. Human RNase inhibitor is an acid protein with molecular weight of 50 kDa. Fu et al. [66] cloned cDNA gene RNase inhibitor (RI) and inserted in retroviral vector pLN CX. The combined vector pLN CX-RI was transfected into retroviral packaging cells, and a clone producing a high titer of virus was obtained. Next, isolated hematopoietic cells from mice bone marrow were infected with virus carrying the pLN CX-RI. After administration of hematopoietic cells, carrying the RI gene were implanted with B16 melanoma cells. The results showed that tumors of control groups became large and well vascularized. In contrast, tumors in mice treated with hematopoietic cells carrying RI gene were small and possessed a relatively low density of blood vessels. The rate of tumor growth inhibition was 47%.

Thus, we can summarize that RNases represent an extensive group of the essential enzymes for a cell which function provide necessary balance between processes of synthesis, operation and destruction of various classes of RNA in the cells on various phases of a cell cycle. Function of these enzymes as the critical epigenetic regulators, in some cases provides signaling tools between cells [1, 70]. Therefore, working out the new ways of influence on RNase activity as well as discovery the new hydrolytic enzymes highly specific on the certain sites of ribonucleic acids, can become the important approach to creation the new directions in cancer treatment.

REFERENCES


