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REVIEW

Cell Adhesion Proteins and α-Fetoprotein. Similar Structural Motifs as Prerequisites for Common Functions

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Abstract—This review summarizes and analyzes data on structural and functional relationships between cell adhesion proteins and α-fetoprotein (AFP), which play an important role in embryo- and carcinogenesis and act in synergism with growth factors. These two groups of proteins are mosaic, multimodular, and polyfunctional, and each of their modules can function independently through binding with its specific membrane receptor. Most cell adhesion proteins contain modules similar to epidermal growth factor (EGF) and also their repeats, which determine the involvement of these proteins in regulation of cell proliferation, differentiation, and apoptosis. These EGF-like modules are found to include short motifs similar to the fragment LDSYQCT of human AFP. Both direct and inverted AFP-like motifs are linked through a consensus octapeptide motif CXXGY/FXGX. Such AFP-like motifs of cell adhesion proteins and the tripeptide RGD found in AFP may be structural prerequisites for common functions of these groups of nonhomologous and unrelated proteins.

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Adhesion of cells to one another and to extracellular matrix (ECM) is important during various physiological and pathological processes, such as embryogenesis, wound healing, immune response, and tumor growth. Cell adhesion provides tissue integrity and interaction and has an influence on regulation of cell proliferation, migration, differentiation, and apoptosis [1-3]. Cell adhesion occurs with involvement of adhesive molecules such as cadherins, selectins, connexins, proteoglycans, immunoglobulins and extracellular matrix proteins. The structure of cell adhesion proteins is characterized by the presence of the tripeptide RGD (arginyl-glycyl-aspartic acid), which is involved in interaction with specific receptors on the cell surface—integrins [4, 5]. Most adhesion proteins are mosaic, multimodular, and polyfunctional proteins. Each of their modules can function independently, possibly through binding with its own membrane receptor. Functionally important modules of these proteins include modules structurally similar to epidermal growth factor (EGF), containing 30-40 amino acid residues (aa), and responsible for the involvement of the proteins in regulation of cell proliferation and differentiation [6].

α-Fetoprotein (AFP) is the major oncofetal protein of all mammals and, possibly, all vertebrates [7-9]. It plays an essential role in embryogenesis and carcinogenesis, being involved in the regulation of cell proliferation, differentiation, and apoptosis. Similarly to cell adhesion proteins, AFP is a mosaic, multimodular, and polyfunctional protein. Despite a rather pronounced similarity between the primary structures of AFP and albumin, which are homologous and 40% identical proteins, and common features of their secondary and tertiary structures, AFP is characterized by some specific features lacking in albumin. Intensive studies during the last decade have revealed in AFP some functionally important sites that are absent in albumin. These findings seem to explain specific functions of AFP [10]. The alignment of primary structures of AFP, albumin, and EGF allowed us to detect in AFP a short motif LDSYQCT (aa 14-20)

Abbreviations: AFP) α-fetoprotein; aa) amino acid; BM) basement membrane; ECM) extracellular matrix; EGF) epidermal growth factor; FAK) focal adhesion kinase; ILK) integrin-linked kinase; IGF) insulin-like growth factor; MAPK) mitogen-activated protein kinase; PDGF) platelet-derived growth factor; PI-3K) phosphatidylinositol-3-kinase; PKB) protein kinase B; RGD) arginyl-glycyl-aspartic acid; RTK) receptor tyrosine kinase; TGF-α) transforming growth factor-α; TNF-α) tumor necrosis factor-α; VEGF) vascular endothelial growth factor.

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which is similar to the part of the EGF receptor-binding site LDKYACN (aa 26-32) but is absent in albumin [11, 12]. The heptapeptide LDSYQCT was prepared by solid-phase chemical synthesis and examined in some biological activity tests. It was established to be a biologically active site of AFP influencing the regulation of lymphocyte proliferation and apoptosis of activated cells [13-15]. We found both direct and inverted forms of motifs similar to the sequence LDSYQCT (AFP-like motifs) in some growth factors of the EGF family [16] and also in EGF-like modules of cell adhesion proteins. Along with detection of the tripeptide RGD [17], this finding suggests structural and functional similarities between cell adhesion proteins and AFP, which are likely to be realized through binding with a common membrane receptor and/or via a similar action mechanism.

Data accumulated during the last decade on the structure, functions, and action mechanism of AFP and cell adhesion proteins are summarized and analyzed in this review, and the structural–functional similarity between these two groups of unrelated nonhomologous proteins is demonstrated.

STRUCTURE AND FUNCTIONAL IMPORTANCE OF CELL ADHESION PROTEINS

Adhesive properties of cells became a subject of intensive studies from the 1970s, when it was first supposed that proteins responsible for cell adhesion should exist on the cell surface [18]. Significant progress in studies on cell adhesion mechanisms was achieved in the 1980s due to detection of the tripeptide RGD in many proteins mediating the interactions of cells to one another and with extracellular matrix [19-21]. This motif was first revealed in fibronectin and later in vitronectin, collagens, thrombospondin, fibrinogen, osteoponin, von Willebrand factor, etc. The tripeptide RGD was shown to provide protein interactions with specific receptors on the cell surface, namely with integrins, and the affinity and specificity of this binding depended on the conformation of the RGD-containing site [22]. There has been significant progress in studies on structure and functions of cell adhesion proteins during the last 10-15 years. This progress was dependent on the development of genetic approaches based on mutant mice carrying deficient genes or mice lacking genes encoding various proteins and their receptors. Important results were also obtained due to the technology of recombinant proteins. The majority of cell adhesion proteins were shown to consist of several distinct modules capable of independent functioning and interacting with cells, ECM proteins, or growth factors [23, 24].

Here we consider the structure and functions of cell adhesion proteins which contain motifs similar to those of AFP. First of all, such proteins include ECM proteins, the majority of which contain EGF-like modules which include direct and inverted AFP-like motifs connected through an octapeptide consensus motif CXXGY/FXGX (table). Revealing of similar motifs suggests the presence of structural prerequisites for common functions of these two groups of unrelated proteins.

Collagens. Members of collagen superfamily are the main components of ECM and are multimodular three-chained proteins produced by homo- or heterotrimerization of different α-chains. At present, different vertebrates are found to have 28 types of collagens (I-XXVIII) formed by 43 unique α-chains [25, 26]. Each collagen type includes a three-chained helix-like collagenous domain, with α-chains consisting of repeats $(G–X–Z)_n$, where X and Z are most often proline and oxyproline, respectively, and specified by a high content of imino acids [26-28]. Collagens also contain two noncollagenous (NC) domains, on the *N*- and *C*-ends of the polypeptide chain (*N*–NC and *C*–NC, respectively). The collagen superfamily forms a great variety of self-organizing molecular and supramolecular structures different in structure, functions, and tissue specificity. These include fibrils, microfibrils, or networks. Homo- or heterotrimers of α-chains act as promoters for triggering their oligomerization during formation of different supramolecular structures [29].

Based on the architecture of these structures, all collagens are classified as follows: fibril-forming or fibrillar (types I, II, III, V, XI, XXIV, and XXVII); network-forming (types IV, VIII, and X); fibril-associated collagens containing interrupted triple helices (FACIT) (types IX, XII, XIV, XVI, and XIX-XXII); anchored fibrillar (types VI and VII) and transmembrane collagens (types XIII, XVII, XXIII, and XXV); collagen-related proteins [30-39].

The type I collagen of fibrillar collagens is best studied: it consists of α1- and α2-chains which form homo-$(\alpha1)_3$ or heterotrimers (α1α1α2) [40, 41]. Noncollagenous *C*-NC-domains of α-chains act as recognition sites, and their interaction starts the formation of homo- or heterotrimeric promoters [25]. These domains inside both α1- and α2-chains form five subdomains and can determine the mode of their trimerization [42]. During the trimerization, disulfide bonds produced between the *C*-NC-domains of α-chains play an important role [43, 44].

Type IV collagen is a typical representative of network-forming collagens [25]; it is a main component of basement membrane (BM) and consists of six types of homologous α-chains (α1-α6) each of which consists of a short (from 13 to 157 aa) cysteine-rich *N*-terminal domain, a long (1400 aa) collagenous domain interrupted by 21-26 noncollagenous domains, and a *C*-terminal noncollagenous NC1-domain (230 aa) [45, 46]. Heterotrimerization of six types of α-chains results in three kinds of the type IV collagen (α1α1α2, α3α4α5, and

Direct and inverted AFP-like motifs of proteins regulating embryo- and carcinogenesis

Protein name and aa numbers in AFP-like and consensus motifs	Amino acid sequence of direct, inverted, and consensus motifs	Protein accession number to Swiss-Prot/TrEMBL database
Human EGF (aa 26-47)	LDKYACN *CVVGYIGE* RCQYRDL	P01133
Mouse EGF (aa 26-47)	LDSYTCN *CVIGYSGD* RCQTRDL	P01132
Human TGF-α (aa 27-48)	EDKPACV *CHSGYVGA* RCEHADL	P01135
Human betacellulin (aa 57-78)	EQTPSCV *CDEGYIGA* RCERVDL	P35070
Human HB-EGF (aa 88-109)	LRIPSCH *CLPGYHGQ* RCHGLTL	Q06175
Human prostaglandin-G/H-synthase-1 (aa 28-49)	LDRYQCD *C*(*T*)*RTGYSGP* NCTIPGL	P23219
Human vaccinia growth factor (aa 45-66)	IDGMYCR *CSHGYTGI* RCQHVVL	P20494
Human epigene (aa 48-69)	LEKAICR *CFTGYTGE* RCEHLTL	Q6UW88
Human neurogenic protein Notch (aa 720-741)	INGYNCS *CLAGYSGA* NCQYKLN	P07207
Human lactadherin (aa 27-48)	FPSYTCT *CLKGYAGN* HCETKCV	Q08431
Cadherin-related tumor suppressor (aa 3989-4010)	LDGAVCQ *CDSGFRGE* RCQSDID	Q14517
Human matrilin-2 (aa 357-378)	DDSYTCE *CLEGFRLA* EDGKRCR	O00339
Human laminin-2 (α-chain) (aa 1467-1489)	LDDYRCT (*A*)*CPRGYEGQ* YCERCAP	P24043
Human coagulation factor VII (aa 65-86)	LQSYICF *CLPAFEGR* NCETHKD	P08709
Human coagulation factor IX (aa 84-105)	INSYECW *CPFGFEGK* NCELDVT	P00740
Human coagulation factor X (aa 65-86)	LGEYTCT *CLEGFEGK* NCELFTR	P00742
Human tomoregulin (aa 68-89)	GESYQNE *CYLRQAAC* KQQSEIL	Q9UK5
Human δ-4-like protein (aa 279-300)	QRSYTCT *CRPGYTGV* DCELELS	Q9NR61
Mouse polydom		
(aa 1270-1291)	LASYRCT *CVKGYMGV* HCETDVN	Q9ES77
(aa 1384-1405)	LNSYSCK *CQPGFSGH* RCETEQP	
(aa 4148-4269)	NGSYVCS *CNPPYTGD* GKNCAEP	

Notes: HB-EGF) heparin-binding EGF. Numeration of aa is given for mature polypeptide chains. Motifs corresponding to the consensus CXXGY/FXGX are italicized.

α5α5α6), which in turn form three kinds of collagen networks inside BM. Under *in vitro* conditions, hexamers can also be produced with involvement of recognition sites located in the *C*-NC-domains of each chain.

Basement membrane also contains the type XV and XVIII collagens, which represent, respectively, chondroitin sulfate and heparan sulfate proteoglycan [47, 48]. BM is a special, highly specialized type of ECM. The structure and functions of BM are different depending on its protein composition [49, 50]. BM components dynamically interact with cells, and this provides transduction of signals determining cell proliferation, migration, differentiation, and survival. The protein components of BM determine its architecture and are also involved in ECM degradation, which occurs during many physiological and pathological processes. ECM degradation leads to generation of biologically active peptides, which regulate various processes during embryo- and carcinogenesis.

Proteolytic fragments of NC1-domains have been found in both vertebrates and invertebrates and display a highly conservative primary structure, i.e. their amino acid composition has been retained during evolution. Endogenous fragments of NC1-domains of α-chains of the type IV, XV, and XVIII collagens (arrestin, restin, and endostatin, respectively) are shown to suppress angiogenesis and are promising for application as inhibitors of tumor growth [51-53]. Endostatin can bind with vascular endothelial growth factor receptor (VEGFR2), and its action seems to be mediated through this receptor. Mouse endostatin inhibits migration and morphogenesis of urinary bladder and kidney epithelial cells and induces their apoptosis [54]. Endostatin is likely to arrest the cell cycle G1-phase via inhibiting synthesis of cyclin D1 [55]. Recent findings have shown that type XVIII collagen is not only a component of BM, but is also actively expressed in the connective tissue of endocardium during early stages of embryogenesis [56]. Type XVIII collagen

and endostatin seem to be involved in regulation of morphogenetic changes during epithelial-to-mesenchymal transition (EMT). EMT is a key process of morphogenesis during which the cell phenotype is changing; highly differentiated and polarized epithelial cells acquire features of mesenchymal cells. This decreases their adhesion and enhances migration in ECM. This phenomenon underlies the formation of a fetal organs and tumor metastasizing. Effects of the α-chain NC1-domains can be mediated by their binding with integrins through both the tripeptide RGD and other motifs [57].

The action of collagens is mediated through their binding with different integrins, such as α1β1, α2β1, αvβ3, α3β1, and αvβ5 [57-59]. Glycoprotein VI receptors in platelets and discoidin domain receptors in different cells can also act as collagen receptors [60].

Laminins. Laminins are heterotrimeric glycoproteins consisting of α-, β-, and γ-chains [61-65]. Combinations of these chains result in different laminin isoforms. For example, laminin-1 consists of α1-, β1-, and γ1-chains, whereas laminin-8 contains α4-, β1-, and γ1-chains, and laminin-10 contains α5-, β1-, and γ1-chains. The *N*-terminal part of the majority of laminins is a short handle consisting of all three types of chains (α, β, and γ) and is involved in the interaction of laminin molecules during their self-assembling [66]. The *C*-terminal part of laminins includes only α-chains, which form a globular G-domain consisting of so-called LG-repeats involved in the interaction with cell-surface receptors. The central part of all laminins contains a long handle formed by the three types of coiled-coil chains [67, 68]. Moreover, laminins also contain EGF-like repeats, which are longer than usual (they have four disulfide bonds instead of three). Some chains of EGF-like modules include direct and inverted AFP-like motifs linked by the consensus motif CXXGY/FXGX (table). All laminins contain the tripeptide RGD, which is mainly exposed upon their proteolytic degradation [69].

Laminins are main components of BM, along with the type IV, XV, and XVIII collagens, nidogens, fibulins, and a heparan sulfate proteoglycan perlican [49, 65]. Laminins undergo a spontaneous self-assembling by a Ca^{2+}-mediated mechanism and produce a polygonal lattice [70]. This is associated with interaction between α-, β-, and γ-chains of the *N*-terminal parts of different laminin molecules, the central parts of which (long handles) bind to cell-surface receptors. The self-assembling of BM components begins from polymerization of laminin-1, which is followed by involvement of other proteins in this process [71]. Laminin-1 is a major contributor to BM formation during early stages of embryogenesis. Studies on mutant mice with deficient genes have shown that other types of laminins, as well as the type IV collagen, nidogens, and perlican, are involved in BM formation during the later stages of embryogenesis [72-74].

Laminin isoforms are tissue specific, and this suggests the difference of their functions in different tissues [75]. Laminins function through binding with receptors on the cell surface, which are mainly represented by integrins of the subclasses α3β1, α6β1, α6β4, α7β1, αvβ1, αvβ3, αvβ5, and α5β1 [69, 70, 76]. Laminin receptors are represented by a transmembrane protein distroglycan and heparan sulfate proteoglycans (sindicans and glypicans), which can function as they are or as co-receptors for integrins [75].

Fibronectin is a multidomain polyfunctional protein consisting of three types of homologous repeats (I-III) [77]. It is a glycoprotein with molecular weight of 220-250 kD, with the polypeptide chain containing two independent adhesive regions. One of them is located in the middle of the polypeptide chain, inside the ninth and tenth modules of the type III, and binds integrins α5β1. This region contains the tripeptide RGD and also the pentapeptide PHSRN, which is also important for functioning of fibronectin. The mutual spatial positions of these peptides and the number of aa between them are very important [78]. The other adhesive region is located on the *N*-end of the polypeptide chain and consists of the type I repeats. Interaction of the *N*-terminal region of fibronectin with integrin receptors α5β1 promotes aggregation with insoluble fibrils and regulates formation of actin complexes and focal contacts, activity of intracellular tyrosine kinases, and cell migration [79]. The *N*-terminal region and the tripeptide RGD compete for binding to integrins α5β1, i.e. can bind to them concurrently.

The soluble form of fibronectin circulating in the blood system can polymerize with production of the insoluble form, which is a component of ECM fibrils. Fibronectin polymerization is a dynamic process, which occurs with involvement of integrins and is triggered by interaction of both the tripeptide RGD and the *N*-terminal region of fibronectin with the cell-surface receptors through their sites of complex formation with the matrix [77-79].

Lactadherin. In humans, this is a milk fat globule-EGF factor 8 (MFG-E8) or mammary gland epithelial antigen BA46. Lactadherin is a major membrane glycoprotein of milk fat globules that is expressed in mammary gland carcinomas [80-82]. Human lactadherin is a 46-kD multimodular protein (387 aa), with an EGF-like module on the *N*-end and two C-like modules (C1 and C2) on the *C*-end, which are specific for coagulation factors V and VIII [83]. The EGF-like module of lactadherin contains short AFP-like motifs. A direct motif FPSYTCT (aa 27-33) in human lactadherin is linked with the inverted motif HCETKCV (aa 32-38) through the octapeptide CLKGYLGN (table). The EGF-like module is rich in cysteine residues and contains the tripeptide RGD responsible for the involvement of lactadherin in cell adhesion [84]. The role of RGD in binding with the cells has been

shown using a recombinant form of lactadherin which binds to human mammary gland carcinoma cells MCF-7 expressing integrin αvβ5 on the surface. The binding is mediated through the RGD tripeptide but not through its analog RGE [85]. Green monkey kidney cells MA-104 which express both αvβ5 and αvβ3 integrins also bind lactadherin. RGD-mediated adhesion of the mammary gland carcinoma ELL-G cells and mouse fibroblasts 3T3-L1 also occurs with involvement of lactadherin [84].

Lactadherin effectively competes with coagulation factors V and VIII for binding with phosphatidylserine-containing membrane [86, 87]. This may be provided by structural homology between lactadherin and the coagulation factors. The ability of lactadherin to bind phospholipids, in particular phosphatidylserine in the cell membrane, through its C-like (or C1 and C2 type F5/8) domains seems to play an important role in intercellular interactions. Lactadherin, which binds through its *N*-end with the integrin receptor of one cell and through its *C*-end with phosphatidylserine of the other cell membrane, acts as a mediator between the two cells [84].

C-Like domains of lactadherin contain a 5.5-kD peptide fragment of 50 aa, which is called medin. Medin is the major component of amyloid fibrils which are formed during a number of neurodegenerative diseases [88]. The octapeptide fragment (NFGSVQFV) of medin can produce typical amyloid fibrils. The hexapeptide NFGSVQ is the smallest peptide fragment capable of producing amyloid fibrils similarly to short amyloidogenic peptides NFGAIL and NFLVH of islet amyloid polypeptide (IAPP) [89]. Lactadherin seems to regulate functioning of aortal elastin fibrils. Medin can mediate the interaction of lactadherin with tropoelastin and participate in adhesion of smooth muscle cells to elastin fibrils of arteries [90].

Matrilins. The structure of matrilins is characterized by the presence of different numbers of EGF-like modules and also subunits containing domains similar to von Willebrand factor A (vWFA) and a series of heptad repeats on the *C*-end of the polypeptide chain [91]. Matrilin-1, -2, and -4 contain two vWFA domains, and matrilin-3 contains one such domain. Due to their heptad repeats, all matrilins can produce coiled-coil α-helical homopolymers, and matrilin-1, -2, and -3 also form heterooligomeric complexes [92-94]. Matrilin-1 is mainly expressed in cartilage ECM, matrilin-3 is expressed in bone tissue, and matrilin-2 and -4 are expressed in many tissues, including connective tissue and subepithelial basal membrane [95]. Using the recombinant form of matrilin-2 expressed by HEK-293 cells, matrilin-2 was shown to interact with other ECM proteins including type I collagen, fibronectin, laminin-1, and fibrillin-2 [96]. Matrilins are supposed to be adaptor proteins promoting interaction of ECM proteins to one another and of proteoglycans with collagens, and also formation of collagen-independent structures. Because matrilin-2 is mainly expressed by mesenchymal cells, prechondrocytes, and preosteoblasts, it is considered to be a marker of cells with a high ability for differentiation [97].

Matrilins may functionally compensate each other; thus, matrilin-4 can function as matrilin-2 in mutant mice deprived of the gene encoding synthesis of the latter [98]. No changes in the structure and development of organs have been revealed in these mice. Immunohistochemical study on the skin of normal and the mutant mice also has not shown changes in expression and accumulation of matrilin-2 ligands, such as the type I collagen, fibrillin-2, and laminin–nidogen complex. Matrilin-2 has the highest molecular weight (107 kD, 956 aa in humans) and contains ten EGF-like modules, two vWFA-like domains, and a unique segment not detected in other matrilins [99]. EGF-like modules seem to be not involved in formation of hetero- and homooligomeric complexes, but during this process the role of cysteine residues is important because they form disulfide bonds [100]. EGF-like modules of matrilins contain both direct and inverted AFP-like motifs (table).

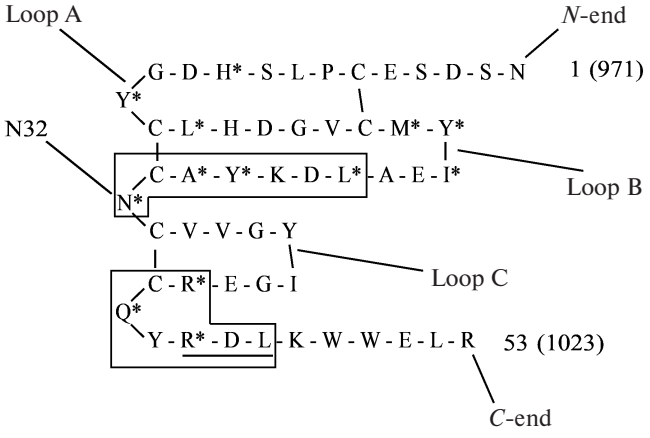
Cadherins. The cadherin superfamily includes 80 proteins that mediate Ca^{2+}-dependent tissue-specific adhesion of cells via their homophilic interaction [101, 102]. Cadherins are membrane proteins consisting of intracellular, transmembrane, and extracellular domains. There are distinguished E-, N-, P-, and M-cadherins, which are mainly expressed in epithelial and nervous tissues, placenta, and muscles, respectively. X-Ray crystallography of cadherins has shown formation of repeated structures in the extracellular domain, which determines specificity of cell–cell interaction [103, 104]. Cadherin-mediated cell adhesion involves some cytoplasmic proteins, in particular catenins. β-Catenins interact with the *C*-terminal part of cadherins and provides their connection with the actin cytoskeleton with involvement of α-catenins, whereas p120-catenins bind to the juxta-membrane domain of cadherins and modulate adhesion and migration of the cells [105]. Interestingly, β-catenins can act as intracellular effectors for the EFG receptor (EGFR), and transactivation of the latter under the influence of p38 leads to phosphorylation of β-catenins and dissociation of their complexes with E-cadherins [106]. Then β-catenins penetrate into the nucleus and induce transcription of genes encoding proteins involved in regulation of the epithelial-to-mesenchymal transition [107]. Such is the role of cadherins in cell morphogenesis and formation of tissue architecture [108].

Cadherins are capable of suppressing tumor growth. Repression of the cadherin expression results in disturbance of intercellular contacts, tumor progression, enhancement of its invasiveness, and metastasizing [109, 110]. Note that two short AFP-like motifs (one is direct, and the other is inverted) linked by the octapeptide CDSGFRGE are found in a protein related to cadherins (table). Possibly due to these motifs, this protein can not

only suppress but also stimulate tumor growth similarly to growth factors TGF-β, which also contain AFP-like motifs [16].

TRANSDUCTION OF A SIGNAL INITIATED BY CELL ADHESION PROTEINS

Direct and inverted AFP-like motifs linked by the consensus motif CXXGY/FXGX were first found by us inside EGF itself and other growth factors of the EGF family (figure). The direct motif LDKYACN (aa 26-32) was shown to be a part of the human EGF loop B containing amino acid residues (L26, Y29, A30, N32) involved in binding to the receptor [16]. The inverted motif RCQYRDL (aa 41-47) is located near the *C*-end of EGF and also contains amino acid residues essential for binding to the receptor (R41, Q43, R45, L47). We supposed that the heptapeptide LDSYQCT in AFP also should be involved in binding to the receptor, which similarly to the EGF receptor could belong to receptor tyrosine kinases (RTK). This hypothesis is confirmed by the experimental findings that the stimulation of some cells by AFP was accompanied by expression of some intracellular effectors of RTK-mediated signaling pathways [111-113]. The consensus motif CXXGY/FXGX resembles the motif GXXG, which promotes interaction of proteins to one another, with DNA, or a ligand due to weak hydrogen bonds between the CαH-group of glycine and CO-group of another molecule [114]. Such hydrogen bonds also arise with involvement of the glycine residue in the repeats $(G\text{-}X\text{-}Z)_n$ upon formation of triple helices of collagens.



Primary structure of human EGF with the mapped AFP-like and consensus motifs. Motifs similar to the heptapeptide LDSYQCT of human AFP are indicated: the direct motif LDKYACN (aa 26-32) and the inverted motif RCQYRDL (aa 41-47). Asterisks indicate amino acid residues involved in binding to the receptor (EGFR). N32 is a hinge region. The numeration is given for a mature molecule (53 aa in total)

It has already been mentioned that many cell adhesion proteins are mosaic and multimodular ones, and each module can act through binding to its own membrane receptor. EGF-like modules of these proteins responsible for their involvement in regulation of cell proliferation, differentiation, and apoptosis are likely to act via the RTK-mediated pathway of signal transduction [6]. It seems that cell adhesion proteins can interact with their classic receptors, integrins, and also with other types of receptors, e.g. RTK. There may exist a crosstalk between the signal transduction pathways mediated by such two groups of receptors. Thus, signals transmitted with involvement of integrins can regulate the activity of growth factor receptors, i.e. integrins can act in coordination with RTK [115]. A specific class of integrins selectively interact with specific receptors of growth factors [116, 117], and this may represent an additional pathway of the integrin-mediated regulation of cell proliferation. The mechanism of RTK-mediated signaling has been described by us in detail [16]. Here we consider the integrin-mediated pathway of signal transduction.

Integrins. Integrins are principal cell receptors mediating adhesion of cells to one another and to ECM proteins [118-120]. Cell adhesion is associated with aggregation of F-actin with specialized structures (focal contacts), changes in pH and Ca^{2+} concentration in the cell cytoplasm, and also in expression of the genes that determine ability of integrins to regulate proliferation, migration, and survival of the cells. Integrins represent a family of receptors which are heterodimeric complexes consisting of noncovalently bound α- and β-subunits. The integrin family is subdivided into subfamilies depending on the type of α- or β-subunits. Each subfamily includes integrins consisting of α- or β-subunits of the same type (e.g. β1). The α- and β-subunits are glycoproteins consisting of globular extracellular, transmembrane, and intracellular domains, and in the majority of integrins the intracellular domain is relatively small and includes about 60 aa. The intracellular domain binds with cytoplasmic proteins and actin cytoskeleton and, thus, is involved in the control of its organization, whereas the extracellular domain interacts with other cells and ECM proteins. For survival and functioning, many normal cells need be attached to adhesion proteins: without such an anchorage they are subjected to apoptosis [116, 121]. This phenomenon is called an anchorage-dependent survival. On the contrary, tumor cells can survive without adhesion to ECM proteins, and this phenomenon is called anchorage independent survival. Various signaling pathways are triggered depending on the ECM composition, participation of specific integrins and intercellular effectors.

According to modern concepts, conformational changes in the cytoplasmic and transmembrane domains of integrins promote signal transduction both from the outside into the cells and in the opposite direction [122]. Activities of the cytoplasmic domains of β1-, β2-, and

β3-subunits of integrins may be sufficient for signal transduction into the cells. The role of these domains has been established experimentally using mutant genes encoding them. For example, mutation in the gene encoding the intracellular domain of the integrin β1-subunit results in disorders in cell proliferation and survival both *in vitro* and *in vivo* [123]. Analysis of crystal structures of the αvβ3-integrin intracellular domain in complex with a cyclic peptide containing the tripeptide RGD and of αIIβ3-integrin complexes with different RGD-containing ligands has shown that the arginine residue is involved in formation of hydrogen bond and the aspartic acid residue is involved in formation of a coordination bond [124, 125]. The glycine residue in RGD is also important for binding with integrins. It is involved in formation of weak hydrogen bonds of the Cα–H...O–C type and determines the interaction specificity [126].

Intracellular effectors. Integrins have been shown to transmit signals into cells via activation of intracellular protein kinases such as focal adhesion kinase (FAK, or pp125FAK) and integrin-linked kinase (ILK) [127, 128]. Adaptor proteins, such as Grb2 and Shc, as well as Nck2, containing SH2- and SH3-domains (i.e. domains homologous to Src, which is a product of chicken sarcoma virus oncogene) are also involved in signal transduction [129, 130].

The ~125-kD nonreceptor tyrosine kinase FAK is one of the key effectors involved in integrin-mediated signal transduction [127]. Activation of FAK is stimulated by clusterization of integrins and occurs with involvement of the NPXY motif of the β1-subunit cytoplasmic domain and functionally active actin fibrils [131, 132]. Downstream key effectors of the FAK-mediated signal transduction are paxillin, protein CAS (Crk-Associated Substrate), and phosphatidylinositol-3-kinase (PI-3K) [133-135]. The binding of fibronectin with NIH 3T3 fibroblasts activates FAK along with the nonreceptor protein kinase c-Src. Their combined action triggers multiple cascade pathways resulting in activation of Ras and as a consequence of ERK2, which is an isoform of a MAPK variety ERK (Extracellular Signal-Regulated Kinase) [136]. These processes involve binding of the adaptor protein Grb2 with FAK at the tyrosine residue Y925, phosphorylation of another adaptor protein Shc at residue Y317, and its binding with Grb2. Note that the signal transduction pathways mediated by Shc and FAK can function independently.

Using primary fibroblasts with a deletion in the gene encoding the cytoplasmic domain of the integrin β1-subunit, it was shown that the effective phosphorylation of tyrosine inside Shc, Shc binding with Grb2, and activation of ERK can occur without FAK [137]. However, in Swiss-3T3 cells expressing B-Raf, FAK intensified and prolonged the integrin-mediated signal which led to activation of ERK with involvement of p130(CAS), Crk, and Rap1. Association of FAK with Src and p130(CAS), phosphorylation of the latter, and activation of Crk were necessary for stimulation of JNK (Jun NH2-terminal kinase) and, as a result, normal progression of the cell cycle G1-phase [138]. The integrin-mediated stimulation of JNK was more effective than its stimulation by growth factors. JNK is another (along with ERK) variety of MAPK capable of penetrating into the cell nucleus and activating in it such transcription factors as c-Jun [139].

Highly conservative positively charged amino acid residues in the β1-subunit transmembrane domain are essential for regulation of transduction of the signal mediated by PI-3K and protein kinase B (PKB/Akt). Mutation in the gene encoding the transmembrane domain of the integrin β1-subunit and resulting in the K756L substitution does not influence their conformation and ability to bind ligands and also phosphorylation of FAK and Src. Activation of paxillin which is downstream intracellular effector for FAK and Src also is not affected, but CAS and Akt are not activated, and the rate of migration and distribution of CD25-β1A (K756L) cells along fibronectin and laminin-1 is decreased [140].

A ~59-kD polydomain integrin-linked kinase (ILK) possessing a serine-threonine kinase activity is another effector protein involved in transduction of integrin-mediated signals [141]. This kinase binds with cytoplasmic domains of integrins through its *C*-terminal domain, which is also involved in interactions with a number of adaptor proteins, such as CH-ILKBP, affixin, and paxillin, capable of binding with actin fibrils [142, 143]. The *C*-terminal domain of ILK also has a protein kinase activity. On the *N*-end of ILK there is an ANK domain containing four ankyrin repeats through which ILK interacts with the protein PINCH (Particularly Interesting New Cysteine-Histidine rich protein). The PINCH protein consists of five LIM-domains, of which LIM1 binds to ILK undergoing conformational changes [144]. Formation of a multicomponent complex PINCH–ILK–affixin–paxillin (the two latter ones are denoted as α-parvin) is an important mechanism of physical binding of integrins with the actin cytoskeleton and plays an important role in the signal transduction from ECM proteins to intracellular effectors [145].

The PINCH protein interacts via its LIM4-domain with the adaptor protein Nck-2 (also known as Nckβ or Grb4), which contains one SH2- and three SH3-domains [146]. The interaction of PINCH with Nck-2 provides crosstalk between the signal transduction pathways mediated by integrins and growth factor receptors. The weak protein kinase activity of ILK can be markedly stimulated by integrin binding with ECM proteins and also by the influence of growth factors [147]. The effects of ILK are mediated by phosphorylation of PKB/Akt, glycogen synthase 3 (GSK3), and myosin light chains [148]. Phosphorylation of PKB/Akt is accompanied by its activation and that of GSK3 is associated with its inhibition. The ILK-mediated signals provide normal functioning

and survival of cells [149, 150]. Hyperexpression of ILK is accompanied by tumor transformation of cells and enhancement of their invasiveness [148].

ROLE OF ADHESION IN CELL MIGRATION, PROLIFERATION, AND APOPTOSIS

Cell adhesion, proliferation, and differentiation. Cell adhesion proteins play an important role during embryo- and carcinogenesis due to their ability to regulate cell proliferation, migration, and apoptosis. The role of cell adhesion to ECM proteins and involvement of integrins in the regulation of the cell cycle and proliferation of normal cells have been shown *in vitro* [151-154]. Cell cycle impairment associated with disorders in signal transduction via the FAK/PI-3K/Akt- and Rac/JNK-mediated signaling pathway was observed in primary embryonic fibroblasts of mice homozygous in a mutant gene encoding the cytoplasmic domain of the integrin β1-subunit [153]. Despite the normal phosphorylation of ERK upon the cell adhesion to fibronectin, its translocation into the nucleus was affected. Expression of an active form of Rac restored the translocation of ERK into the nucleus, and this suggests an important role of Rac in a signal transduction leading to MAPK activation. Hence, for the normal progression of the cell cycle the interaction between the integrin β1-subunit and the protein Rac is necessary.

The action of cell adhesion proteins, similarly to that of AFP [16], is coordinated with the synergistic action of growth factors. The combined effect of signal transduction pathways mediated by integrins and growth factor receptors involves FAK and ILK. In spite of recognition of the leading role of growth factors in regulation of the cell proliferation and differentiation, the role of ECM during these processes seems to be no less, and, possibly, even more important. In some cases, cell adhesion to ECM proteins is required for stimulation of cell proliferation by growth factors. The adhesion to ECM proteins increases the cell susceptibility to signals initiated by growth factors and also determines the character of these signals [155]. For example, proliferation and functioning of vascular endothelial cells are regulated not only by mitogenic factors (such as vascular endothelial growth factor VEGF) but also by adhesion to extracellular matrix proteins. Adhesion to ECM stimulates proliferation, migration, and survival of endothelial cells under the influence of VEGF, and this effect is mediated by integrins αvβ3 and α3β1 [156-159]. In endothelial cells of umbilical cord vessels, the interaction between the receptor VEGFR2 and integrins αvβ3 is necessary for the complete phosphorylation of VEGFR2. This activates FAK and stimulates the stress-activated protein kinase-2/p38 (SAPK-2/p38), which is a third variety of MAPK (in addition to ERK and JNK) [158]. Adhesion of endothelial cells to ECM proteins and their migration are mediated by αvβ3 integrins. Using neutralizing antibodies, interaction of lactadherin with integrins was shown to regulate the VEGF-dependent phosphorylation of Akt and neovascularization of tissues [160].

The integrin-dependent adhesion of endothelial cells to fibronectin provides tyrosine phosphorylation in FAK and paxillin and activation of MAPK, but does not cause autophosphorylation of RTK. EGF stimulates autophosphorylation of EGFR and does not cause FAK activation. However, adhesion to fibronectin increases activation of MAPK under influence of the growth factor [156]. Moreover, adhesion to fibronectin increases activation of JNK in the presence of tumor necrosis factor-α (TNF-α) and thus significantly contributes to cell cycle regulation.

Phosphorylation of FAK is also stimulated by platelet-derived growth factor (PDGF), and this is accompanied by association of FAK with PI-3K, enhancement of hepatocyte proliferation, and expression of type I collagen [161]. Inhibition of FAK in liver stellate cells affects their migration, adhesion, and PDGF-mediated activation of PI-3K [162]. These data suggest the involvement of PDGF in regulation of migration, adhesion, and proliferation of these cells, realized via FAK/PI-3K-mediated signal transduction pathway.

Interaction between the β1-subunit and insulin-like growth factor 1 (IGF-1) receptor regulates adhesion and migration of human multiple myeloma cells [163]. The interaction of IGF-1 with its receptor induces phosphorylation of IGF-1R and association of IGF-1R with the integrin β1-subunit that increases adhesion of MM.1S and OPM6 MM cells to fibronectin. This is accompanied by activation of PI-3K(p85), Akt, and ERK. IGF-1 also enhances polymerization of F-actin, phosphorylation of FAK and paxillin, and strengthens their interaction with the β1-subunit.

Coordinated signal transduction by growth factor receptors and integrins regulates cell proliferation also via activation of cyclin-dependent kinases, which control the cell cycle G1-phase. This is realized mainly through regulation of key cyclins of the G1-phase such as D1 and cyclin-dependent kinase inhibitors such as p27 (kip1) [164-166].

Cell adhesion and apoptosis, multidrug resistance. The binding of integrins with ECM proteins promotes anchorage-dependent cell survival both *in vitro* and *in vivo*. Many cells, e.g. fibroblasts and endothelial cells, undergo apoptosis because of disorders in their adhesion to ECM [167, 168]. The anchorage-dependent cell survival has been termed *anoikis*, which means homelessness. Adhesion of human endothelial cells to fibronectin or vitronectin decreases their sensitivity to apoptosis and increases their survival. The suppression of cell apoptosis and activation of their proliferation are accompanied by stimulation of MAPK [169, 170]. Activation of signal transduction pathways leading to an increase in cell sur-

vival can be mediated by ERK1 and ERK2 and enhanced expression of c-Flip, which is an endogenous antagonist of caspase-8. *Anoikis* caused by disorders in cell adhesion to ECM can be caused by binding of Fas to its ligand Fas-L and activation of the Fas-mediated signal transduction pathway [171]. Combination of effects of different ECM proteins provides achievement of maximum effect, i.e. they act in cooperation.

Survival of endothelial cells and their acquisition of angiogenic phenotype can be mediated by the αvβ3 integrins. Antibodies against αvβ3 integrins or antagonistic peptides suppress adhesion of endothelial cells to ECM and *in vivo* inhibit angiogenesis stimulated by growth factors or tumor growth via induction of cell apoptosis in the newly generated vessels [172]. Tumor necrosis factor TNF-α and γ-interferon suppress activation of the αvβ3 integrins and cell adhesion mediated by them and also decrease cell survival [173]. Interestingly, the reinforced vasculo- and angiogenesis and 80%-lethality during embryogenesis were observed in mutant mice lacking the genes encoding the αv-chains of integrins [174]. Integrins of the αvβ3-subclass also induce *anoikis* of human intestinal carcinoma cells. Cells insensitive to *anoikis* are characterized a decreased synthesis of the integrin αv-subunit [175]. Genetic deficiency leading to the absence of β3- and β5-integrins was associated with increased tumor growth and angiogenesis. This suggests that such integrins are not essential for neovascularization [176]. Angiogenesis induced by hypoxia and vascular endothelial growth factor is increased in the absence of β3-integrins. However, the level of other integrins is unchanged, whereas the level of the VEGFR2 receptor is increased. These findings may be explained by compensation of functions of β3- and β5-integrins by other integrin subclasses or VEGF.

Anoikis can be regulated by the integrin β1-subunit, which stimulates activation of PI-3K and protein kinase B (PKB/Akt) [177]. Dephosphorylation of FAK and PKB induced by contraction of collagen fibrils leads to apoptosis of fibroblasts. Dephosphorylation of PKB and induction of apoptosis of fibroblasts can be also caused by suppression of ILK activity [178]. Adhesion of small-cell lung cancer cells to fibronectin, laminin, and type IV collagen with involvement of the β1-subunit causes resistance to apoptosis induced by standard chemotherapeutical agents, such as etoposide, *cis*-platinum, and adriamycin [179]. At that cell adhesion stimulates tyrosine kinase activity in cells both treated and untreated with etoposide, which prevents the agent-caused activation of caspase-3 by the agent and, as a result, suppresses cell apoptosis.

Inhibition of T-lymphocyte apoptosis during their activation is also mediated by the cell adhesion with involvement of β1-integrins. In activated peripheral blood T-lymphocytes and tumor cells of the Jurkat line, binding of type I collagen with integrins of the α2β1-subclass markedly decreased apoptosis induced by activation of Fas and by caspase-8 [180]. The suppression of apoptosis was accompanied by a decrease in binding of annexin V and DNA fragmentation, did not need protein synthesis, and depended on activation of the signal transduction pathway mediated by ERK1 and ERK2.

In many tumor cells, the gene encoding tumor suppressor p53 is inhibited simultaneously with disorders in adhesion to ECM. Cell adhesion to fibronectin is associated with an increase in their survival and in activation of FAK. In the absence of FAK or adhesion to ECM, tumor cells undergo apoptosis through the p53-dependent mechanism [82]. Embryonic fibroblasts of homozygous mice deficient in gene encoding FAK were more sensitive to apoptosis induced by TNF-α [181]. In these cells, there was no expression of IRS-1 (Insulin Receptor Substrate-1) and activity of nuclear factor NF-κB was suppressed, and this was accompanied by a decrease in the level of an antiapoptotic protein Bcl-xL. Thus, FAK protects cells from apoptosis, and this is regulated by phosphorylation and activation of nuclear factor NF-κB and interaction between antiapoptotic signaling pathways and mechanisms mediated by growth factors with involvement of PI-3K, ERK1, and ERK2.

Recent studies have shown that the integrin-mediated signal transduction is an important mechanism underlying development of multidrug resistance. Attachment to ECM provides tumor cell resistance to many chemotherapeutic agents acting via induction of apoptosis [182, 183]. The detailed role of integrins in the development of multi-drug resistance remains unclear, but there are data indicating interdependence between the integrin-mediated pathways of signal transduction and signals controlling the cell cycle and apoptosis. The relation of this phenomenon with changes in synthesis of some classes of integrins has been also shown. For example, kidney carcinoma cells with an acquired drug resistance are characterized by changes in synthesis of β1-integrins [184]. The acquisition by mammary gland adenocarcinoma MCF-7 cells of multiresistance to antitumor drugs (doxorubicin, vincristin, taxol, and mitoxantrone) is accompanied by decrease in the sensitivity to *anoikis* and a significant increase in invasiveness *in vitro* [185]. The multidrug resistant MCF-7Dox cells displayed a markedly lowered expression of β1-integrins. It seems that disorder in adhesion of the MCF-7 tumor cells to ECM with involvement of β1-integrins decreases their sensitivity to apoptosis. Interestingly, the acquisition of multi-drug resistance by fibroblasts is accompanied by a decrease in synthesis of αvβ3-integrins, lowering of collagenase MMP-2 activity, and a decrease in the invasiveness and metastasizing of tumor cells [186]. These findings confirm a pro-apoptotic role of this subclass of integrins. It seems that αvβ3-integrins mediate transduction of signals caused by disorders in cell adhesion to ECM and leading to enhancement of apoptosis.

α-FETOPROTEIN

Structure of α-fetoprotein. Similarly to all secreted proteins, AFP is synthesized as a precursor from which a mature molecule is produced during processing by cleavage of the signaling peptide and glycosylation. The polypeptide chain of human AFP includes 591 aa [187-189]. AFP is a ~69-kD glycoprotein containing 3-5% of carbohydrates, and 5.4% of the aa are cysteine residues half of which are double-cysteines, i.e. follow one after another. Cysteine residues are highly conservative. They are present in sequences of all proteins of the albumin family, to which AFP belongs, and are found in all biological species. The polypeptide chain of AFP contains 32 cysteine residues; the first two do not produce disulfide bonds, whereas the other residues form 15 regularly arranged intramolecular disulfide bridges, which form a cystine frame of the molecule.

The primary structures of AFP and albumin display up to 40% identity, have similar α-helical secondary structures (67 and 50% of α-helical regions in AFP and albumin, respectively), and contain no β-structure. These proteins also have a similar spatial organization: they include three homologous domains (I-III), each of which consists of two globular subdomains (IA, IB, IIA, IIB, IIIA, IIIB) [189, 190]. Domains I and III have a rigid and compact tertiary structure and are linked by a proteolytically labile and flexible domain II [191]. However, despite a rather high similarity of the primary and spatial structures of AFP and albumin, the functional properties of these two related proteins are different. Although both albumin and AFP can bind and, possibly, transport different hydrophobic ligands, AFP has also some specific functions; in particular, it can regulate cell proliferation, differentiation, and apoptosis [10]. The specific functions of AFP seem to depend on the presence in its primary structure of a number of short motifs, each of which can be responsible for some particular function of AFP.

AFP contains more than 20 short sequences similar to functionally important regions of certain physiologically active proteins, including polypeptide growth factors [10]. Some of these sequences have been synthesized as separate peptide fragments and studied using different biological activity tests. One of such biologically active region is represented by the heptapeptide LDSYQCT (aa 14-20). It has been shown that this peptide in dose-dependent fashion inhibits lymphocyte proliferation in patients with acute and chronic lymphatic leukemia with a low sensitivity to cytozar, i.e. the heptapeptide increase an antiproliferative activity of this drug, possibly, by overcoming a resistance to it [192]. At concentration of 10^{-7}-10^{-9} M this heptapeptide moderately stimulates proliferation of non-activated lymphocytes and significantly inhibits proliferation of lymphocytes activated by phytohemagglutinin (PHA) [193]. The inhibition of the PHA-activated proliferation is 40% and does not depend on the peptide concentration. The peptide probably inhibits *in vivo* lymphocyte proliferation, which is increased in autoimmune diseases, not affecting the normally proliferating cells [194, 195].

This heptapeptide significantly decreases expression of the late activation antigen HLA-DR and induces expression of Fas-antigen (CD95) in cell culture from patients with infectious allergic myocarditis with naturally activated lymphocytes. The increase in the number of CD95+ lymphocytes indicates that LDSYQCT induces apoptosis of activated cells, because this process depends on the Fas/FasL-cellular interaction [195]. Thus, LDSYQCT is a biologically active region of AFP possessing immunomodulating properties and ability to regulate cell proliferation and apoptosis. At that the heptapeptide stimulates proliferation of non-activated lymphocytes and down-regulates proliferation of activated lymphocytes, inducing their apoptosis. The enhancement of cell sensitivity to cytozar seems to be caused by induction of their apoptosis, which is mediated by cooperation of signals transmitted by different receptors, including RTK and integrins.

Similarly to ECM proteins, domain II of AFP contains the tripeptide RGD (aa 253-255 in human AFP), which also may act as a signaling adhesive site [17]. Interestingly, a direct motif similar to the motif LDSYQCT, namely IMSYICS (aa 266-272), has been also found in domain II of human AFP.

Regulation of cell proliferation, differentiation, and apoptosis. The ability of the intact AFP molecule to regulate proliferation, differentiation, and apoptosis of both embryonic and tumor cells, including lymphoid and epidermal cells, fibroblasts, liver, placenta, ovary, and uterus cells has been described [196, 197]. AFP is shown to both up- and down-regulate cell proliferation and differentiation. The inhibitory effect of AFP is mainly manifested on proliferation of estrogen-sensitive cells [198-200], whereas AFP stimulates proliferation of estrogen-independent cells. Thus, AFP dose-dependently stimulates proliferation of human embryonic fibroblasts from different organs [201]. AFP increases the number of polygonal cells in the mouse placenta and also increases synthesis of DNA, and this indicates proliferation of these cells [202].

The ability of AFP to stimulate proliferation of tumor cells has been shown using different experimental models *in vitro*. AFP pronouncedly increased proliferation of mouse hepatoma H-22 and Ehrlich ascites carcinoma cells and human hepatocellular carcinoma BEL-7404 cells [197, 203]. The stimulation by AFP of cell proliferation is tumor-specific. Thus, in the same system the same concentrations of AFP increase proliferation of the BEL-7404 cells and have no influence on proliferation of HL-60 cells [203]. Moreover, antibodies against human AFP suppress the growth of BEL-7404 cells and do not affect proliferation of HL-60 cells.

In cultures of human hepatoma HepG2, lymphoblastoma MT4, and Jurkat lymphoma cells and of mouse fibroblastoma L929 cells high concentrations of AFP (>100 μg/ml) inhibited, whereas low concentrations of AFP stimulated proliferation of the same cell lines. Interestingly, removal of secreted cytokines and growth factors from the cultural medium of human myeloblastoma U-937 and mammary gland carcinoma MCF-7 cells was associated with an increase in the cell sensitivity to inhibitory effect of high doses of AFP [201]. Hence, cytokines and growth factors decrease the inhibitory effect of high doses of AFP.

Synergistic effects of AFP and growth factors similar to those of growth factors and cell adhesion proteins have been shown in a number of studies. For example, purified AFP does not stimulate mitogenesis in porcine granulosa cells but nearly two-fold strengthens stimulation of proliferation of these cells by EGF and IGF [204, 205]. In the same cell culture, AFP suppresses the TGF-α-stimulated synthesis of estradiol. The ability of umbilical cord blood to stimulate proliferation of mammary gland medullar carcinoma cells was 75% lowered upon the removal of AFP by affinity chromatography and increased 1.5-2.0-fold on addition of PDGF (10 ng/ml) into the medium [206]. At that, the effect of PDGF was 56% decreased on removal of AFP from the medium, i.e. the purified preparation of AFP increased the ability of PDGF to stimulate cell proliferation. These data indicate synergistic effects of low concentrations of AFP and growth factors on cell proliferation.

AFP also modulates the activity of VEGF, which is a major mitogen inducing vasculogenesis (the *de novo* generation of blood vessels) and angiogenesis (the generation of blood vessels from pre-existing capillaries) in endothelial cells. At concentrations close to physiological (10-100 ng/ml), AFP stimulated proliferation of endothelial cells in microvessels of human placenta and uterus and increased the ability of VEGF to stimulate proliferation of vascular endothelial cells. In the concentration of 100 ng/ml, AFP also induced phosphorylation of ERK1 and ERK2 [112]. Therefore, AFP is suggested to act via binding to a membrane receptor possessing tyrosine kinase activity, because both ERK1 and ERK2 are key effector proteins of the signaling pathway initiated by RTK. This hypothesis is also supported by data obtained in culture of proliferating HeLa and BEL-7402 cells [114, 207].

Stimulation of cell proliferation by low concentrations of AFP can be associated with inhibition of apoptosis. Thus, under the same conditions, AFP can stimulate proliferation of the HL-60 cells and suppress their apoptosis [208]. Susceptibility to senescence of HL-60 cells during their normal *in vitro* reproduction correlates with their sensitivity to apoptosis and the loss of ability to express receptors for AFP. Therefore, inhibition of apoptosis is supposed to be mediated by AFP binding with a cell-surface receptor.

AFP is responsible for resistance of hepatocarcinoma HepG2 cells to cytotoxic effect of TNF-α [209]. At concentrations of 10-100 μg/ml, AFP prevents the TNF-α-induced death of mouse hepatocellular carcinoma cells due to binding with TNF-α and inhibition of TNFR1 receptor-mediated signal transduction [210]. TNF-α-induced cell death can be caused by inhibition of nuclear factor NF-κB activity [211]. NF-κB can induce expression of genes that control synthesis of cell survival factors, such as Bc1-X_L and XIAP, which results in suppression of apoptosis of the cells. In addition, the *AFP* gene seems to be a target gene for NF-κB, because inhibition of NF-κB is accompanied by suppression of AFP synthesis.

In contrast, inhibition of cell proliferation in the presence of high concentrations of AFP can be caused by induction of apoptosis. High concentrations of AFP (100-200 μg/ml) induce apoptosis in different lines of human tumor cells [212, 213] by a mechanism independent of Ca^{2+} and protein kinase activity, and this does not require RNA and protein synthesis. An addition of AFP into the tumor cell cultures caused release of cytochrome *c* from mitochondria into the cytoplasm, formation of apoptosomal complexes, and activation of caspase-3 and -9.

AFP receptor and a possible mechanism of its action. The ability of AFP to regulate cell proliferation, differentiation, and apoptosis seems to be mediated through receptors on the cell surface. An AFP receptor was first detected on the surface of human mammary gland carcinoma MCF-7 cells [214]. Later, cells of human lung, prostate, ovary, and uterine cancer, mouse T-lymphoma YAC-1, rat Morris 777 hepatoma, and rhabdosarcoma were also shown to have the ability to recognize and specifically bind AFP [215-222]. Receptors for AFP were also found on the surface of immunocompetent cells [214, 218].

In total, three types of AFP receptors have been revealed on the surface of different cells: one of them is highly specific with a low binding capacity, the second has a low affinity and a high binding capacity, and the third one has medium values of affinity and binding capacity. Different receptor types seem to play different functional roles, because high affinity receptors are saturated at physiological (for adults) concentrations of AFP (10 ng/ml), whereas low affinity receptors are saturated at its high concentrations. The molecular weight of the receptor determined by SDS-PAGE under reducing conditions is 62-65 kD [218].

However, the primary and spatial structure of the receptor for AFP and structure of the gene encoding its synthesis are still unclear. Mechanism and stages of the intracellular transduction of the signal initiated by AFP binding with the receptor are also unknown. Nevertheless, there are some hypotheses about possible mechanisms of AFP action based on experimental data on its intracellular effectors [113, 114]. The AFP-stimulated proliferation of hepatocellular carcinoma BEL-

7402 cells is accompanied by an increase in expression of some protooncogenes, such as *c-fos*, *c-jun*, and *N-ras*. Protein products of the *c-fos* and *c-jun* genes are components of the transcription factor AP-1, which is activated by MAPK and induces transcription of a number of genes controlling synthesis of proteins involved in cell proliferation and differentiation. The gene *N-ras* controls synthesis of the protein p21(ras), which is an effector of RTK-mediated signal transduction. AFP increases synthesis of p21(ras) and mutant tumor suppressor p53. Stimulation of cell proliferation and increase of p21(ras) and the mutant p53 are also observed in HeLa cell culture [207]. At concentrations close to physiological (from 10 to 100 ng/ml), AFP stimulates proliferation of endothelial cells of microvessels of human placenta and uterus and enhances the activity of VEGF [112]. The AFP-stimulated cell proliferation is accompanied by elevation of phosphorylation of two MAPK isoforms, ERK1 and ERK2.

These data, along with revealing of the structural motif LDSYQCT similar to a part of the EGF receptor-binding site [223], suggest that AFP, similarly to proteins of the EGF superfamily, acts via binding to receptor possessing tyrosine kinase activity. AFP may either act autonomously, through binding with its own receptors, which may possess tyrosine kinase activity or may modulate growth factors through binding with their receptor. This is supported by the earlier mentioned ability of AFP to bind to the three types of receptors with different functions. Because AFP contains the tripeptide RGD, it is supposed to be involved in binding with integrins and to act through binding with them. AFP can promote the binding of growth factors or cell adhesion proteins with their receptors and thus strengthens their effects. This can be a demonstration of coordinated actions of different receptors, and their ligands can regulate cell proliferation, differentiation, and apoptosis, amplifying and/or compensating the effects of each other.

Even in the late 1980s, it was suggested that EGF-like modules of ECM proteins should initiate transduction of signals which control proliferation and differentiation of cells [6]. We revealed short heptapeptide motifs similar to the sequence LDSYQCT of human AFP (aa 14-20) in some proteins involved in regulation of embryo- and carcinogenesis, namely in growth factors of the EGF family and also in EGF-like modules of cell adhesion proteins. These AFP-like motifs found in both a direct and inverted form are linked by the consensus motif CXXGY/FXGX. The direct and inverted AFP-like motifs in EGF itself are shown to contain amino acid residues essential for binding with its receptor belonging to the RTK class [16]. We believe that EGF-like modules of cell adhesion proteins can also act through binding with RTKs and interact with them with involvement of the AFP-like motifs.

We proposed earlier that, along with binding to its own receptor, AFP could also bind with growth factor receptors [16]. Based on the revealing in AFP of the tripeptide RGD and its analogs, AFP is also supposed to bind with integrins, similarly to cell adhesion proteins [17]. The ability to bind with integrins can be the basis for the involvement of AFP in cell adhesion and underlie regulation of cell apoptosis and drug resistance.

Regulation of cell proliferation, differentiation, and apoptosis during embryo- and carcinogenesis seems to be realized due to combined action of growth factors, cytokines, and other regulatory proteins. Receptors of AFP, growth factors, and cell adhesion proteins mediating these processes seem to function in coordination and, thus, provide synergistic effects of these proteins.

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