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EPITHELIAL-MESENCHYMAL TRANSITION IN CARCINOGENESIS

Summary. *Transient non-oncogenic EMT is a normal cellular program that initiates cell migration during embryogenesis to direct organ development. In differentiated tissues EMT directs wound healing, regeneration and remodeling. EMT — the epithelial-to-mesenchymal transition is the basis platform of the tumor microenvironment. EMT causatively binding with the tumor progression, by which are modulated the migrational, invasive and metastatic potentials of the tumor cells. Therefore, EMT is the crucial microenvironment metastatic niche for the final cancer cell aggressiveness. Moreover, EMT underlies cancer stem cell induction and also controls tumor drug sensitivity modulating the response of cancer cells to chemotherapy. The earlier event of EMT me-tastatic cascade is the epigenetic deregulation of the E-cadherin-beta-catenin adhesive signaling. The epigene-tic regulation of EMT might be considered as the target for a new cancer epigenetic therapy strategies.*

INTRODUCTION

Epithelial-mesenchymal transition (EMT) is highly conserved cellular program causing transformation of polarized adhesive epithelial cells in mobile, morphologically changed stem mesenchymal cells [1]. This important process upon the physiological conditions takes place in the early stages of embryogenesis and morphogenesis [2]. Later, EMT has been identified as mechanism of launching of invasion and metastasis of cancer cells [3]. Key signal pathways responsible for the induction of EMT in embryogenesis are involved in the process of tumor invasion and metastasis (Fig. 1). Among them it has been showed that Wnt-β-catenin signaling, TGF-β signaling and Notch signaling in the late stages of tumor progression promote EMT in cancer cells providing them with capability of invasion [4].

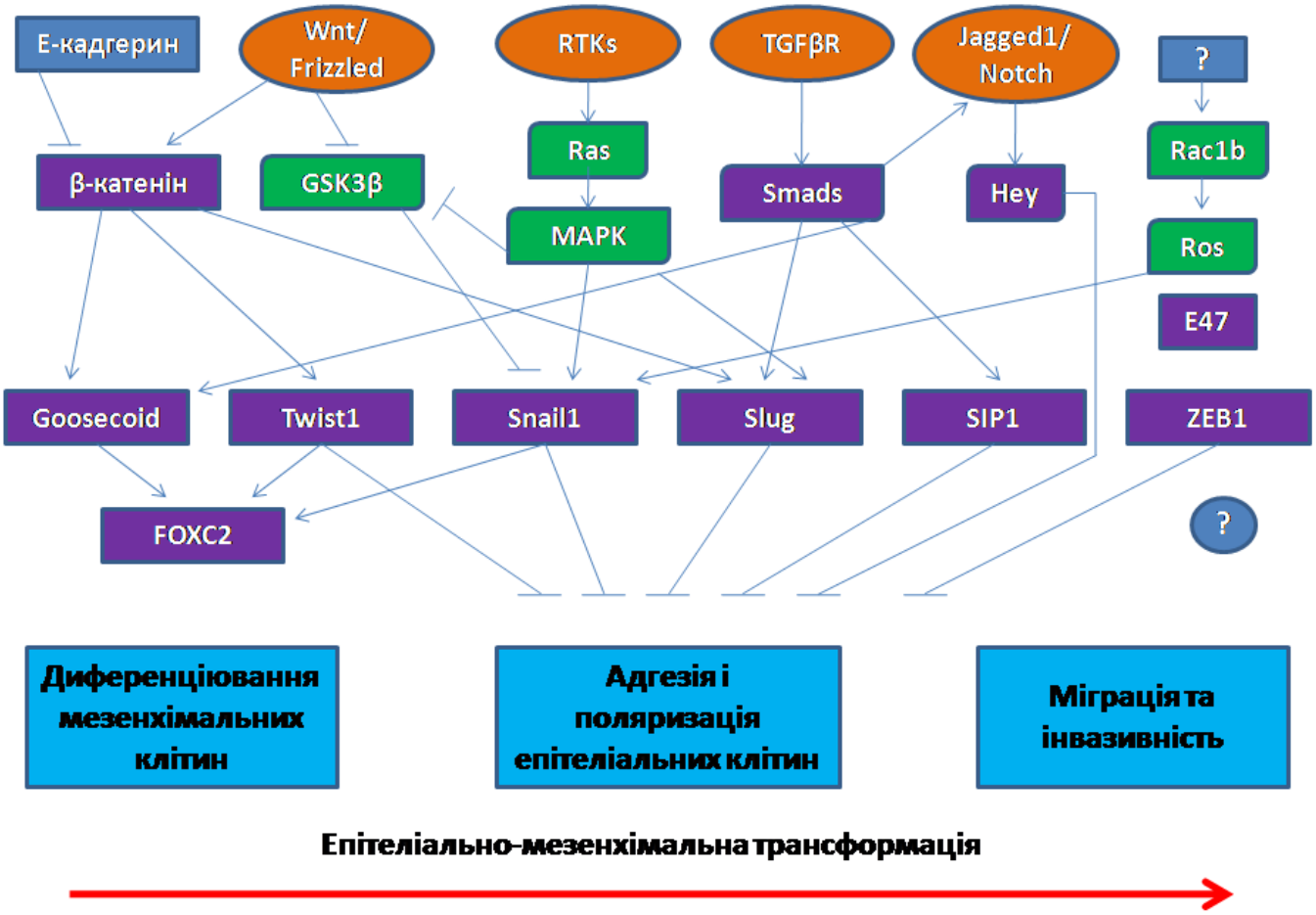

Fig. 1. Signal molecular pathways and transcription factors, which regulate EMT program in cancer cells [4]

Диференціювання мезенхімальних клітин — Differentiation of mesenchymal cells

Адгезія і поляризація епітеліальних клітин — Adhesion and polarization of epithelial cells

Міграція та інвазивність — Migration and invasiveness

Епітеліально-мезенхімальна трансформація — Epithelial-mesenchymal transformation

Dynamic connections between adhesion regulators and nuclear regulators take important place in EMT programming at carcinogenesis. It is canonical Wnt signal pathway and loss of E-cadherin – key marker of epithelial cells – that are involved in nuclear activation of beta-catenin, which, in turn, induces the EMT-associated transcription factors, such as Snail, Slug, Zeb1, Zeb2, Twist1 [5]. Moreover, TGF-β signal pathway also activates EMT-inducing transcription factors, including Slug, SIP1 and Goosecoid in combination with activation of Smads [6, 7]. Notch signal pathway is activated indirectly due to TGF-β-induced EMT [4].

Most of the markers, which are expressed alone in epithelial (cytokeratin 18, mucin Muc-1, desmoplakin) or mesenchymal (vimentin, fibronectin) cells, cannot be fully adequate for prognosis of EMT in cancer progression. Tumor cells passing the stage of EMT, probably, are identified by expression of genes of above mentioned transcription factors – molecular EMT inductors – *Snail, Slug, Zeb1, SIP1 (Zeb2), Twist1, Goosecoid,* which can gain direct diagnostic importance.

In cellular EMT mechanisms, epigenetic regulation takes key place and is combined with disorder of epigenetic control, first of all, of Wnt-beta-catenin signal pathway, to be exact – its aberrant activation in carcinogenesis that accompanies metastatic potential of tumor cells. With activation of Wnt-beta-catenin signal pathway is directly associated aberrant promoter hypermethylation of E-cadherin gene at different types of neoplasms, including leukemia [8, 9]. It causes the suppression of its transcription and, as the result, loss of adhesive molecular intercellular signaling, to nuclear translocation and activation of beta-catenin in structure of Wnt-beta-catenin signal pathway instead [10]. Such “switching” of epithelial E-cadherin-beta-catenin adhesive complex on the nuclear Wnt-beta-catenin signal pathway of proliferation of mesenchymal stem cells may be

considered key phenomenon of EMT in evolution of tumor-associated stem cells. Such signal pathways and their regulation associated with emergence of tumor stem cells attract attention as potential targets in strategy of modern cancer therapy.

1. HETEROGENEITY OF CELLULAR COMPOSITION OF TUMOR

Important distinguishing feature of tumors is heterogeneity of cellular composition [11]. Despite the fact that subtypes of normal cells inside organ are morphologically similar, neoplastic cells inside tumor are essentially different. For instance, they can be heterogeneous by size, having large number of nuclei, different by form and capability of specific staining. Morphological heterogeneity is important criterion of classification of tumors. It can be presumed that it appears due to the functional heterogeneity in tumor genome. For instance, different chromosome abnormalities are present in all types of cancer cells [12], and aneuploidy in due time has been considered sufficient for explanation of genetic instability without presence of genetic mutations [13]. Changes in chromosomes actually can encompass not only millions of nucleotides, but also single functional regions of chromosomes, complicating thereby the search for the specific mutations for those or other types of tumors. EMT is new established phenomenon in mechanism of heterogeneity of tumors.

2. CHARACTERISTIC OF MORPHOLOGICAL SIGNS OF EPITHELIAL AND MESENCHYMAL CELLS

Epithelial and mesenchymal cells are two mostly widespread types of cells in mammals. Epithelial cells are characterized by cohesive interaction between cells, formation of continuous layers of cells, presence of three membrane domains – apical, lateral and basal, presence of dense contacts between apical and lateral domains, apical-basal distribution and polarity of various organelles and components of cytoskeleton [14]. Mesenchymal cells differ from epithelial cells, first of all, in the way that they are not able to form structured continuous layer of cells, have no well-defined apical-basal polarization, are mobile cells, which can acquire invasive properties [15]. During embryonic development and morphogenesis, the epithelial transformation of pool of cells in mesenchymal due to the EMT phenomenon takes place [2] that assists the formation of three-layer embryo during the gastrulation. EMT is a key in such histogenetic processes, as formation of heart, organs of musculoskeletal system and most of peripheral nerves [16]. In some cases, inverse transition – mesenchymal-epithelial transition can occur.

Aberrant cellular program causing the EMT phenomenon in carcinogenesis is characterized by strengthening of only certain aspects of full EMT program in the process of embryonic development [14].

3. LOSS OF EPITHELIAL PHENOTYPE AND ROLE OF E-CADHERIN

Protein E-cadherin is important for the formation and maintenance of embryonic epithelial cells. Suppression of its expression is essential part of some morphogenetic processes inside embryo, most of which are conditioned by EMT [17].

Transcription factors Snail and Slug are present in non-differentiated mesoderm and tissues, where EMT proceeds, to be exact: neural crest and primitive streak. These data correspond with data that transcription factor Snail suppresses the E-cadherin gene in sites, which pass the EMT during the embryonic development [5].

E-cadherin is a key adhesive molecule, which is calcium-dependent transmembrane glycoprotein and is contained in most of epithelial cells both of embryo and adult organism. E-cadherin acts as suppressor of the development of cancer tumors, setting the molecular barrier for their invasion. Transcription of E-cadherin gene in the most of tumors is absent, and its renewal in cells of carcinomas *in vitro* is sufficient factor for decrease of aggressiveness of these cells [18].

Promoter of E-cadherin structurally consists of non-methylated CpG-islands and E-box [19, 23], which stipulate two types of its regulation: epigenetic and transcriptional. E-box has sites for recognition by transcription factors and contains *5'-CACCTG* sequence that closely coincide with DNA-binding site of transcription factor Snail. One of the main mechanisms in carcinogenesis is transcriptional inactivation of CpG-promoter of gene E-cadherin at its aberrant hypermethylation.

Thus, E-cadherin is responsible for the maintenance of epithelial phenotype of cells. Catenins -β, -α and -γ are molecules of adhesive complex, which bind the E-cadherin protein with actin filaments of cytoskeleton of cell (Fig. 2) [19]. In epithelial layers, intercellular contacts are formed due to the participation of molecules of E-cadherin that forms clusters and small adhesive complexes, which assist the formation of desmosomes [20]. At aberrant promoter methylation of E-cadherin gene, the binding with beta-catenin, which acquire properties of transcription factor, is lost. This process causes the disintegration of adhesive complex.

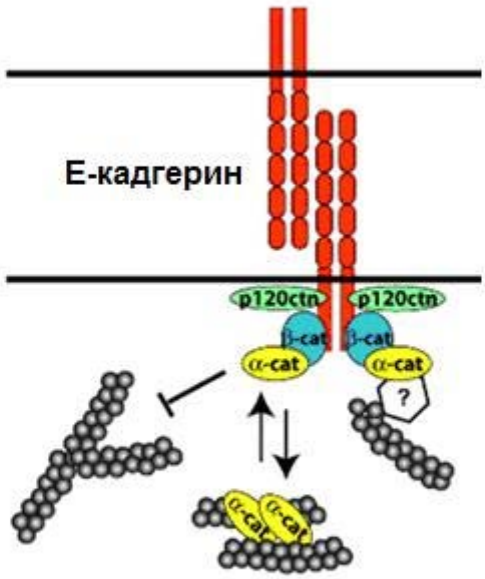

Fig. 2. Structure of E-cadherin-beta-catenin adhesive complex [19]

It has been showed that *AKT* gene described as retroviral oncogene with serine-threonine-kinase activity [21] regulates E-cadherin on the level of mRNA and protein. On the molecular level, *AKT* is activated in epithelial cells and has such consequences for E-cadherin gene, as suppression of its transcription and accumulation of its residue in perinuclear organelles [22]. At the same time, two important sites of *E-box* of promoter, which suppress the expression of E-cadherin gene, take place: *Ets* sites and palindrome *E-pal*. It is interesting that acting as a repressor of transcription of E-cadherin gene, Ets factors, among which is AKT, also participate in regulation of key mediators of invasiveness, such as matrilysin, matrix metalloproteinase, collagenase, heparinase, and urokinase [23].

Research, in which hyperexpression of mutant form of AKT gene in cancer cells has caused the EMT activation and suppression of E-cadherin expression, finally has proved the idea that EMT is initiated with the help of *AKT* gene [24]. EMT activated with the help of AKT enables decrease of cellular adhesion, loss of cellular contacts, morphological changes, loss of apical-basal polarization of cells, activation of mobility of cells, changes in delivery of specific proteins (production of metalloproteinases). For instance, desmoplakin protein, which participates in formation of desmosomes, is being internalized, and vimentin (protein of intermediate filaments of cytoskeleton) present in the most of mesenchymal cells, is activated. Thus, AKT proteins (1-3), which participate in many cellular processes, including regulation of cellular cycle, cellular proliferation, survival of cells, can also be cellular modulators of EMT due to the suppression of E-cadherin-dependent adhesiveness of tumor cells.

4. REGULATION OF EXPRESSION OF E-CADHERIN GENE DUE TO TRANSCRIPTION FACTORS

Factors of two types interact with E-cadherin gene: type “loop-helix-loop”, which includes E12/E47, Twist1 (Twist), Twist2 (Dermo1) [4], and type “zinc finger”, which includes Snail family (Snai1, Slug or Snai2) [5] and Zeb family (Zeb1, Zeb2, which is also called SIP1) [25]. These transcription factors bind *E-boxes* inside promoter of E-cadherin gene and thereby suppress transcription of this gene. The mentioned factors are important for induction of EMT and negative regulation of E-cadherin gene. They launch canonical Wnt-beta-catenin signal pathway, which is key factor in EMT activation.

4.1. Transcription factor Snail (Snail1)

Key transcription factors of Snail family (Snail1, Slug) are strong repressors of transcription of E-cadherin gene [5]. Lines of cancer cells without E-cadherin expression produce large amount of Snail protein, and transfection of E-cadherin-positive lines with the assistance of *Snail* caused the induction of EMT and expression of mesenchymal markers [26]. Moreover, epithelial cell lines, which express Snail, acquire phenotype of fibroblasts with carcinogenic and invasive properties. Thus, Snail transcription factor can be considered probable marker of malignant phenotype [5].

In addition to E-cadherin, Snail transcription factor is involved in negative regulation of other epithelial markers, such as desmoplakin, mucin Muc-1 and cytokeratin 18 (Fig. 3). Vimentin and fibronectin, markers of mesenchymal cells, are also regulated with the assistance of Snail. Thus, Snail transcription factor has series of additional targets in regulation of EMT [27]. Recently, series of negative regulators of Snail transcription factor have been described. For instance, activation of *p53*

that is stipulated by activation of *miRNA-34a/b/c* genes, suppresses the Snail expression. Suppression of *miRNA-34a/b/c*, in turn, activates Snail transcription factor and assists the process of metastasis [28].

4.2. Transcription factor Slug (Snail2)

Slug is a transcription factor of Snail family, which is involved in EMT and is essential for the migration of neural crest from neural tube and early mesoderm from primitive steak of chicken embryo [29]. Role of Slug transcription factor in EMT lies in the loss of cell adhesion and also increase of mobility of epithelial cells. However, it has been demonstrated that expression of Slug transcription factor at melanoma is not connected with decrease of expression of E-cadherin, canonical Slug target in EMT. Expression of exogenous Slug in melanocytes and melanoma cells *in vitro* suppresses the expression of E-cadherin and strengthens the expression of N-cadherin, providing migration of cells. Level of expression of Slug transcription factor is the highest at the beginning of progression of melanoma [6].

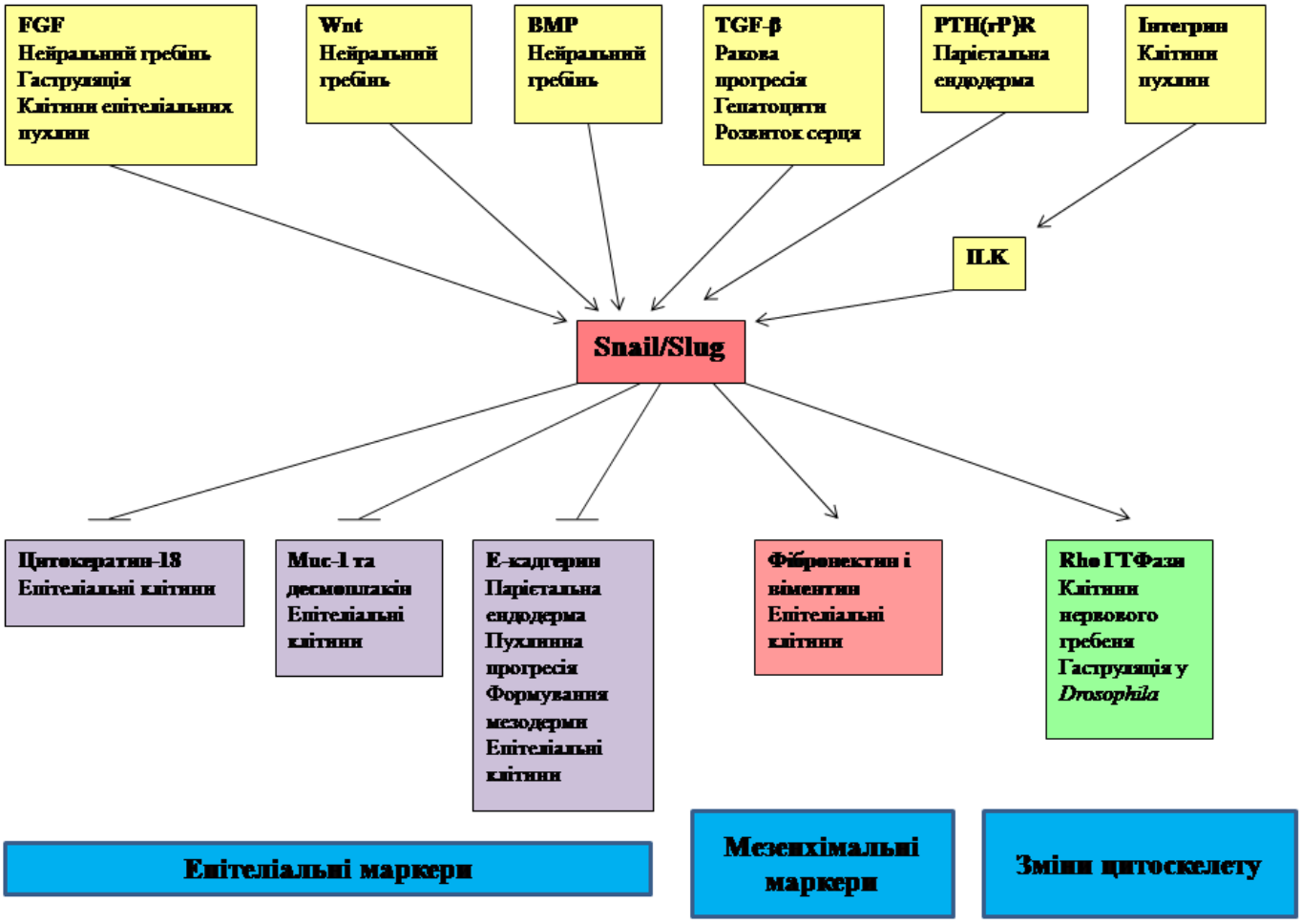

Fig. 3. Snail genes encompass central positions in induction of EMT upon physiological and pathological conditions [27]

Нейральний гребінь — Neutral crest

Гаструляція — Gastrulation

Клітини епітеліальних пухлин — Cells of epithelial tumors

Нейральний гребінь — Neutral crest

Нейральний гребінь — Neutral crest

Ракова прогресія — Caner progression

Гепатоцити— Hepatocytes

Розвиток серця — Development of heart

Злиття неба — Fusion of palate

Парієтальна ендодерма— Parietal endoderm

Інтегрин— Integrin

Клітини пухлин— Tumor cells

Цитокератин-18— Cytokeratin-18

Епітеліальні клітини— Epithelial cells

Muc-1 та десмоплакін — Muc-1 and desmoplakin

Епітеліальні клітини — Epithelial cells

E-кадгерин — E-cadherin

Парієтальна ендодерма — Parietal endoderm

Пухлинна прогресія — Tumor progression

Формування мезодерми — Formation of mesoderm

Епітеліальні клітини — Epithelial cells

Фібронектин і віментин — Fibronectin and vimentin

Епітеліальні клітини— Epithelial cells

Rho ГТФази Rho GTPases

Клітини нервового гребеня — Cells of neural crest

Гаструляція у Drosophila— Gastrulation in Drosophila

Епітеліальні маркери— Epithelial markers

Мезенхімальні маркери— Mesenchymal markers

Зміни цитоскелета — Changes of cytoskeleton

4.3. Transcription factor Zeb1

Transcription factor Zeb1 participates in activation of EMT and inhibition of ageing processes [30]. In association with transcription factor Twist, Zeb1/Twist has dualistic impact on the cancer progression by simultaneous inhibition of ageing, which is caused by activation of oncogenes, and launching of EMT.

4.4. Transcription factor Zeb2 (SIP1)

Transcription factor SIP1 is another one *E-box*-binding protein of "zinc finger" type functioning as strong repressor of E-cadherin gene. High level of *SIP1* expression is marked in cells, which have aberrant hypermethylation of E-cadherin gene [31]. Hyperexpression of *SIP1* decreases proliferative possibilities of cell by suppression of cyclin D3 [32]. Moreover, SIP1 can modulate TGF-β signal pathway, which is known to be involved in EMT activation.

4.5. Transcription factor Twist1

For the first time, Twist1transcription factor has been identified in Drosophila as one of genes essential for the formation of mesoderm, tissue differentiation and development of dorsoventral axis in the early embryogenesis [33]. Gene *Twist1* codes transcription factor of the type "loop-helix-loop" (bHLH) [34]. Expression of *Twist1* is connected with aggressiveness of various tumors. Twist1 participates in initiation, progression and metastasis of cancer cells [35]. *Twist1* can be activated at caused by oncogenes ageing and apoptosis [36], increased resistance of cancer cells to the chemotherapy [13]. It is interesting that both Twist1 and Twist2 suppress induced by oncogenes and p53-dependent cellular death. Gene *Twist1* indirectly impacts the p53 through the suppression of expression of *ARF* in modulating of ARF/MDM2/p53 signal pathway [36]. Thus, Twist1 and Twist2 activate EMT and aggressive cellular migration in epithelial cells. It is supposed that Twist protein can help cancer cells avoid defense programs of organism. It has been showed that induction of EMT via expression of *Twist1* and *Snail* in epithelial cells causes the increase of population of stem cells with high level of CD44 expression and low level of CD24

expression, while isolated epithelial-stem-like cells express the endogenous EMT-associated factors, including Twist1, Snail, Slug and FOXC2 [37].

Recent studies have showed that there is probable molecular mechanism, in which Twist1 transcription factor suppresses expression of E-cadherin gene and assists the migration of cancer cells, invasion and metastasis [34]. Authors have characterized Twist1-associated protein complex and have demonstrated that Twist1 indirectly or directly interacts with components Mi2/nucleosome and deacetylase (Mi2/NuRD) protein complexes, including MTA2, Rb-associated protein 46 (RbAp46), Mi2 and histone deacetylase 2 (HDAC2). Twist1 mediates association of these protein complexes to the promoter of E-cadherin gene and suppresses thereby its activity and expression through chromatin-dependent mechanisms.

5. SIGNAL PATHWAYS PARTICIPATING IN EMT

The main difference between normal development and pathological process is that molecular events are strictly regulated in time and space during the development and differentiation of cell, while these events in carcinogenesis become stochastic. However, phenomenon of EMT and signal pathways, activation of which accompanies its induction, becomes priority in mechanism of malignant transformation.

Key carcinogenic signal pathways, in which are involved, in particular, Sarc, Ras, α-, β-integrin, TGF-β, Wnt/β-catenin and Notch cause the directed induction of EMT and decrease of regulation of cellular adhesion, in particular expression of E-cadherin. Moreover, is has been showed recently that activation of phosphatidylinositol-3′ -kinase pathway PI3K/AKT is also main sign of EMT [2]. Such massive activation of signal pathways, which is constitutively connected with massive activation of the related transcription factors in induction of EMT and suppression of expression of E-cadherin (Snail, Slug, Twist 1,2, Zeb 1,2, etc.), gain particular importance as biomarkers in phenomenon of EMT [24].

5.1. TGF-β signal pathway and some ways of its activation

In consequence of TGF-β activation of EMT, epithelial cells loose adhesion and polarity and acquire mesenchymal phenotype that accompanies resistance of cancer cells [38]. Transforming factor of growth beta (TGF-β) plays dualistic role in progression of breast cancer. TGF-β acts as suppressor and activator of tumors [39, 40]. In normal, it is expressed in cells of mammary gland. Expression of gene TGF-β increases during the pregnancy [41]. Resistance of cancer cells is stipulated by TGF-β-mediated blocking of cellular cycle and apoptosis [40]. TGF-β signaling participates in suppression of cellular proliferation, however, this factor can assist the progression of tumor, if cells acquire resistance before suppressor effect of TGF-β [42]. *In vivo* Ras-transformed cells gradually acquire phenotype of fibroblasts that correlates with transition from paracrine to autocrine activation of TGF-β signaling pathway [43]. MAPK signaling activated due to Ras oncoprotein has important value for EMT and metastasis, while PI3K, other Ras effecter, first of all induces the suppression of apoptosis via activation of TGF-β signaling [44]. Stable activation *Raf* in culture of epithelial cells of kidney MDCK (Madin — Darby canine kidney cells) causes EMT due to autocrine expression of TGF-β [42]. *In vivo* studies on the model of skin cancer have showed double-value role for TGF-β: it suppresses proliferation in the early hyperplastic stage and shows the enhancer function in progression of invasiveness of carcinoma [45].

After EMT, mesenchymal cells acquire signs of TISC (tumor initiating stem-like cells) — signs of neoplastic phenotype by mechanism of self-recovery, important for maintenance of tumor progression. Induction of EMT due to TGF-β is connected with acquisition of these features and activation of Snail1 and Nanog transcription factors at breast cancer [3, 46]. It should be noticed that expression of both *Snail1* and *Nanog* is higher in mesenchymal cells. In mesenchymal cells, the inhibition of Snail1 causes the loss of Nanog and shortening of number of TISC features. With reduction of Snail in mesenchymal-Snail1-shRNA cells, decrease of regulation of *Snail1* correlates with decrease of expression of Nanog and CD44 [3]. Suppression of Snail1 alone is not sufficient factor for inhibition of initiation of carcinogenesis, but it actually causes the decrease of speed of growth of tumor *in vivo* [46].

Activation of specific transcription of genes due to β-catenin is also coordinated by TGF-β signaling (Fig. 4). Genes are activated differently: either only due to Wnt signaling, or in complex with TGF-β. The highest level of transcription of genes is observed, when they are activated due to Smads, TCF/LEF and β-catenin [47].

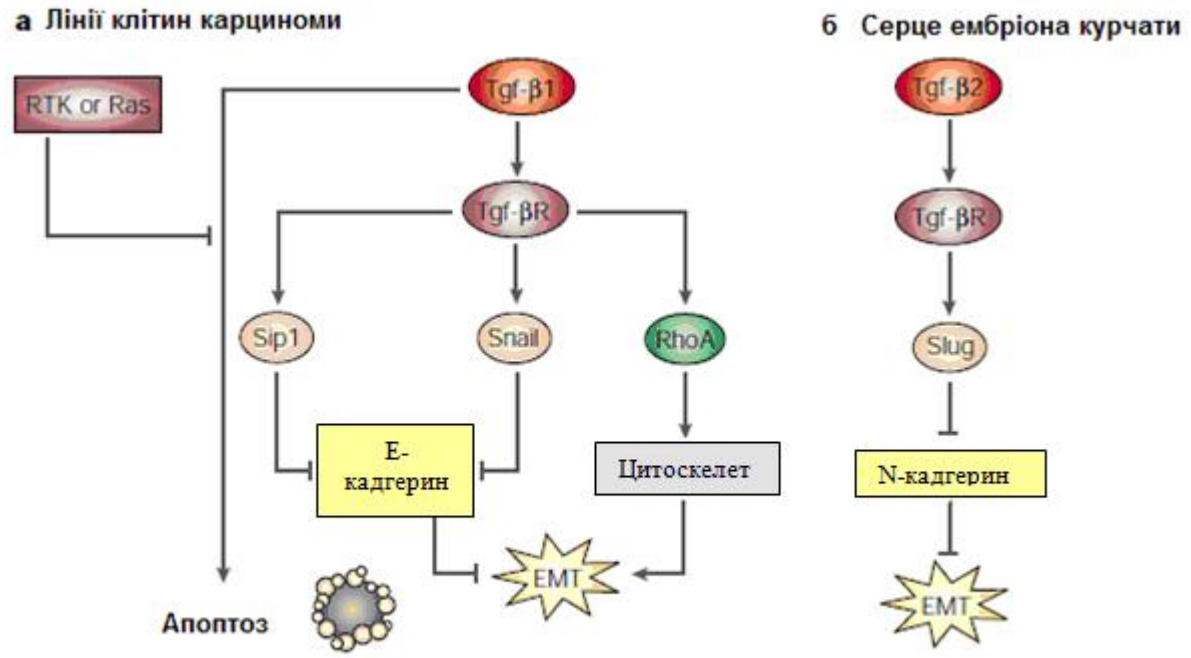

Fig. 4. Dualistic role of TGF-β in initiation and progression of carcinoma [48]

Лінії клітин карциноми — Lines of carcinoma cells

Е-кадгерин — N-cadherin

Цитоскелет — Cytoskeleton

Апоптоз — Apoptosis

Серце ембріона курчати — Heart of chicken embryo

N-кадгерин — E-cadherin

5.2. Wnt signal pathway

Wnt signaling is one of the strongest signal pathways of control of genetic programs of embryonic development and homeostasis of stem cells in ontogenesis. Canonical Wnt signaling plays important role in maintenance of various embryonic stem cells that is also typical concerning phenotype of cancer-associated stem cells. If deregulated, such signal pathway causes serious defects of development and pathologies, including cancer pathologies, therefore, according to the modern data, Wnt signaling acquires key significance in target medicine of cancer stem cells [49].

Signal is initiated by Wnt ligands: in human genome are 19 Wnt ligands [50]. Secretion and posttranslational modification of Wnt ligands is achieved due to multidomain complex and adjacent molecules, such as Wintless (Wnts) [51].

Wnts bind and activate specific receptors of members of Frizzled and LRP families. When interacting, inhibitors of Wnt-receptors secret Frizzled-related proteins (sFRP), Dickkopfs (Dkk), Wnt inhibiting factors (WIF1) that have relation to natural down-regulation of Wnt signal pathway [52]. Wnt signaling starts on the plasmatic membrane via binding of Wnt ligand with Fz receptor and LRP. This signal is transmitted in cytoplasm through Dsh/Dvl and causes the suppression of beta-catenin/Axin/APC/GSK3β complex and proteasome degradation of beta-catenin [53].

5.3. Canonical Wnt/β-catenin signaling

The main function of canonical Wnt signaling is control of stability of β-catenin [53]. The latter has double function (adhesive molecule and signal as transcription factor) and can form stable complexes with cellular adhesive molecules, such as E-cadherin, γ- and α-catenins and, thus, be mediator between transmembrane E-cadherin and cytoskeleton [54]. If Wnt signal is absent, β-catenin, which has not been bounded in complex with cadherin, is captured by multicomplex of proteasome degradation that contains GSK3β, Axin/Conductin, APC, and via its sequential phosphorylation and ubiquitination of N-terminal sequence becomes target of proteasome degradation [55]. If Wnt ligand is absent, β-catenin phosphorylates due to complex, which includes scaffold-proteins Axin, Apc and Gsk3β. Phosphorylated β-catenin is recognized by E3-ubiquitin ligase β-TrCP and is the object of proteasome degradation. In contrast to this, if Wnt ligands are

present, complex of proteasome degradation is being destroyed via phosphorylation of LRP5/6 and binds Axin with LRP that prevents degradation of β-catenin [56]. Free β-catenin, acquiring function of transcriptional co-factor, delocalizes in nuclear, binds with Tcf/Lef family of transcription factors and replaces natural transcriptional inhibitor Groucho in further target activation of Wnt/beta-catenin signaling and its target genes (*Ras, c-myc, Okt4, cyclin-D, etc.)*, which assist maintenance of phenotype of cancer-associated stem cells [57].

Thus, acquired mutations, which activate *Wnt* signaling, can possess carcinogenic activity, while mutations, which are associated with suppression of *Wnt* signaling, cause the suppressor activity in carcinogenesis. For instance: mutations in genes, which control stability of β-catenin, such as *APC*, genes of axin and E-cadherin, cause the development of different types of cancer by carcinogenic activation of β-catenin and Wnt-β-catenin signaling [58, 59].

Tcf/Lef1 are key transcriptional factors regulating expression of genes after activation of Wnt signaling in carcinogenesis, to be exact of oncogenes *Ras*, *c-myc*, *Okt4* and *cyclin-D* in nucleus, which are directly associated with phenotype of cancer stem cells. Thus, carcinogenic function of β-catenin as co-factor is reduced to the activation of genes *Tcf/Lef1* of transcriptional factors in β-catenin-Wnt signaling pathway. Transcription factor EMT, such as Snail, Slug cause carcinogenic activation of β-catenin [60]. On the other hand, no changes of regulation of *Snail* and *Slug* after hyperexpression of *Lef* in epithelial cells was marked that also connects Snail and Slug only with transcriptional activation of β-catenin [61]. However, interesting interrelation arises between TGF-β and Wnt signaling pathways through the role of *Snail* in suppression of expression of E-cadherin [62]. Activation of canonical Wnt signaling pathway is caused by accumulation of β-catenin at alteration of its proteasome degradation in cytoplasm in carcinogenesis, by tyrosine-kinase activation [63] for the specific binding with TCF/LEF by transcriptional factors in translocalization to the nucleus and regulation of expression of target genes of Wnt signaling pathways [64]. On the other hand, high level of E-cadherin directs β-catenin for the formation of adhesive complexes on the membrane of cells [54]. Thus, TGF-β signaling assists the mediated activation of β-catenin-Wnt signaling pathway by specific impact on decrease of expression level of gene of E-cadherin via maintenance of expression of Snail factor (Fig. 4) [24]. It explains why in TGF-β-mutant rats, which have weak Wnt signalization, the functioning of E-cadherin is impaired [65].

5.4. Initiation of Wnt/β-catenin signaling pathway

Initiation of Wnt/β-catenin signaling pathway occurs on the cytoplasm membrane. Binding of secretory protein Wnt1 with complex Fz/LRP causes the phosphorylation of LRP 6 with the help of kinase Gsk3β and casein kinase CkIγ, which probably are necessary for affiliation of Axin with phosphorylated residues of LRP 6. It has been showed that phosphorylated ligands LRP 6, Dvl, Fz, Axin and Gsk3β co-localize in signalosomes [66]. Fz, Dvl and Axin are essential signal molecules for LRP 6 phosphorylation with the help of Gsk3β [67]. At absence of secretory activation of Wnt signaling, phosphorylated β-catenin is a target of proteasome degradation due to complex, which contains Axin, APC, and Gsk3β. In general, it provides the maintenance of model, in which secretory protein Wnt1 is bounded with Fz (Frizzled)-proteins, and Gsk3β can phosphorylate attached to LRP 6 residues of Axin that stipulates the binding with scaffold-proteins and inactivation of complex of proteasome degradation of β-catenin (Fig. 5). Such model confirms hypothesis of staged stowage of molecular components of Wnt signaling pathway [68].

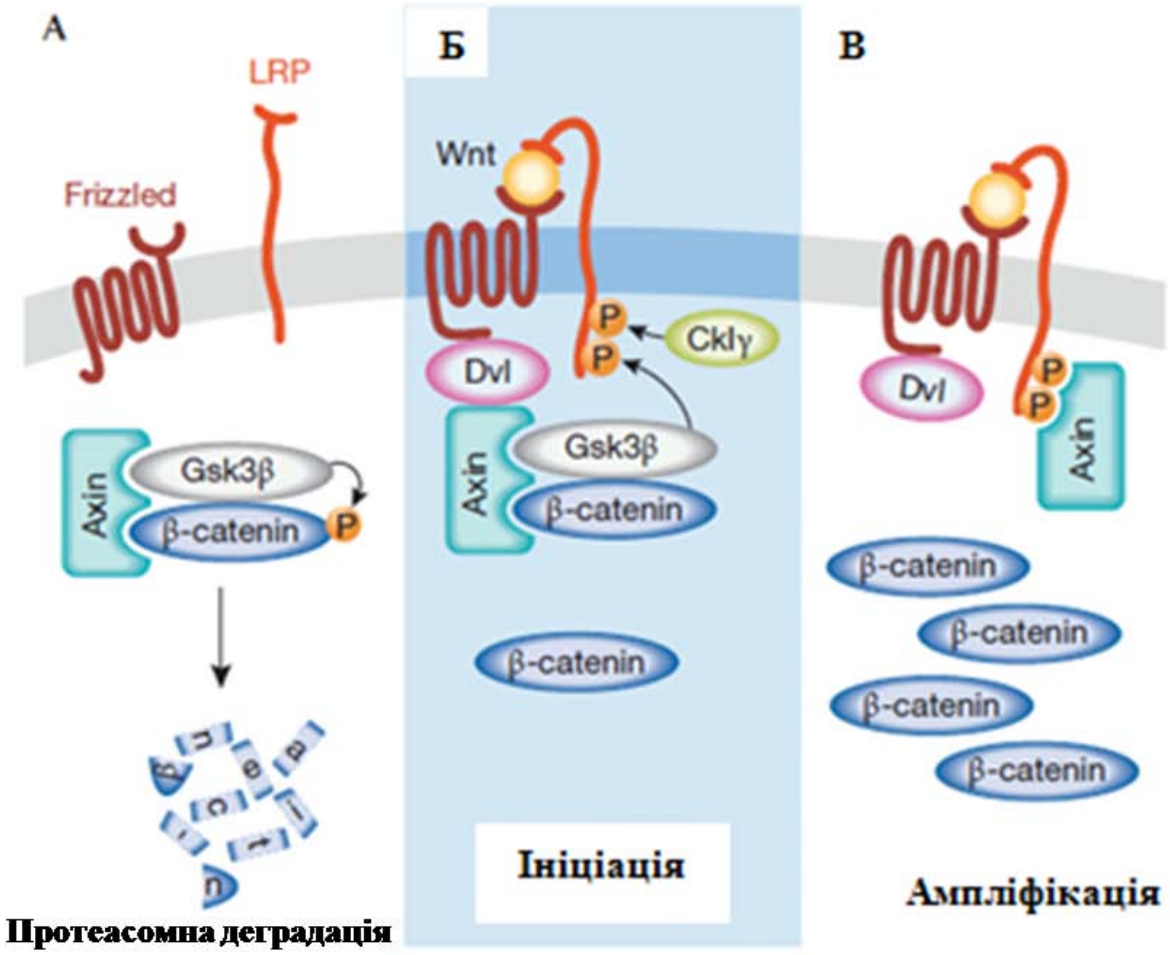

Fig. 5. Model of activation of Wnt/β-catenin of signaling pathway [67]

Протеасомна деградація — Proteasome degradation

Ініціація — Initiation

Ампліфікація — Amplification

It has been showed that cells, which accumulate β-catenin in nucleus, can represent migrating stem cells and be heterogeneously distributed both in primary tumor and in metastases [69]. Axin2 degrades fast after mitosis in such cells; it is presumed that it influences the regulation of β-catenin signaling and stimulates new phase of mitotic cycle [67].

Activation of tyrosine kinases can stimulate β-catenin signaling in nucleus [2]. Structural and functional unity of cadherin-catenin complex is regulated by phosphorylation. Serine-threonine-kinase phosphorylation of β-catenin by Gsk3β and CkIγ kinases correspondingly causes the increase of resistance of E-cadherin-β-catenin complex. However, tyrosine-kinase phosphorylation of β-catenin due to cytoplasm kinase (Fer3) prevents the binding of β-catenin with α-catenin [70], while phosphorylation of β-catenin due to Scr or epidermal growth factor (EGF) prevents binding of β-catenin with E-cadherin. Thus, activation exactly by tyrosine kinases is associated with loss of E-cadherin-mediated intercellular adhesion and increase of level of free cytoplasm β-catenin with further translocalization to nucleus and acquisition of carcinogenic function [71].

CONCLUSIONS

Progression of cancer is the last phase of development of tumor via malignization, in which the final role is played by stages of invasion and metastasis of tumor cells. Role of stem cells in invasive-metastasing cascade of tumor is beyond the question. To understand the mechanism of induction of circulating tumor-associated stem cells in invasive-metastasing phenotype means to find biological ways to overcome progression of cancer and antitumor cell therapy, targets of which become not stem cells itself, but cellular molecular mechanisms, which accompany their induction. Conception of authors lies in the idea that phenomenon of EMT in microenvironment of tumor is a key program to invasive-metastasing phenotype of cancer cells, and, therefore, as likely as not it can be considered specific logotype of cancer progression. EMT mechanism in

carcinogenesis includes two phenomena: morphological and signal. First one is associated with morphological transformation of epithelial cells in mesenchymal cells that takes place at decrease of expression of epithelial markers (E-cadherin, cytokeratin, desmoplakin, etc.) and induction of mesenchymal markers (Snail, Slug, Twist, N-cadherin, etc.). Second phenomenon of EMT lies in maturing of mesenchymal cells to the tumor-associated stem cells that is accompanied by activation of series of signal pathways of stem cells (Wnt/β-catenin, TGF-β, Notch, etc.). Disorders of controlled balance of epithelial cells in microenvironment of tumor and, as the result, induction of EMT in initiation of invasive-metastasing potential of cancer cells can be the target of cellular regulation in antitumor therapy. For instance, loss of expression of E-cadherin – key molecule of intercellular adhesive signaling – and "switching" to the β-catenin-Wnt signaling is regarded as fundamental event in phenomenon of EMT at tumor progression. Thus, with loss of adhesiveness of tumor cells, probably, is associated with induction of invasive-metastasing potential of cancer cells that can open the new way to regulation of these processes, in particular, epigenetic, in antitumor therapy.

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