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Invasion Mechanisms of Bladder Cancer: A Molecular Review

Mesane Kanserinde İnvazyon Mekanizmaları: Moleküler Bakış

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ABSTRACT

Bladder cancer (BC) is a very common cancer and it has high mortality rates, especially in late stages. BC is considered as a different disease in various stages and grades. Non-muscle-invasive BC and muscle-invasive BC have different properties. There are some prognostic factors for progression and recurrence rates of BC and we have some risk assessment methods. These factors are based on clinical findings and histopathological properties. We do not know which factors and molecular processes are effective in the invasion mechanism. In this review, we summarized possible invasion mechanisms of BC.

Keywords

Bladder cancer, invasion, metastasis, molecular

ÖZ

Mesane kanseri farklı evrelerde farklı birer hastalık gibi kabul ve tedavi edilir. Her evre tekrarlama ve ilerleme açısından farklı riskler taşır. Günümüzde bu tekrarlama ve ilerleme risklerini gösteren değişik hesaplama sistemleri tanımlansa da altta yatan moleküler, genetik ve çevresel faktörler net olarak gösterilememiştir. Mevcut risk hesaplama sistemleri klinik, makroskopik ve patolojik değerlendirme temellerine oturmaktadır. Derlemede özetlemeye çalıştığımız olası invazyon mekanizmaları aydınlatıldıkça prognostik yeni moleküler faktörlerin tanımlanması, hatta bu temelde yeni tedavi metodlarının geliştirilmesi mümkün olacaktır.

Anahtar Kelimeler

Mesane kanseri, invazyon, metastaz, moleküler

Introduction

Urothelial tumors are classified as papillary and nonpapillary (solid) tumors. Histopathologically, more than 90% of bladder cancers (BCs) are urothelial (transitional) cell carcinomas (UCC or TCC). At the time of diagnosis, about 75% of BCs are non-muscle-invasive bladder cancer (NMIBC) and remaining 25% are muscle-invasive bladder cancer (MIBC). Phenotypes and genotypes of these cancers in various stages are different. In this context, 30% to 50% of NMIBCs recur after transurethral resection of the primary tumor, and 10% to 20% progress to MIBC. Many of high-grade carcinomas (including carcinoma in situ) invade the bladder wall and may metastasize to other sites. Additionally, nearly 50% of patients with MIBCs have occult distant metastases at the time of diagnosis (1,2). Recently, in the literature, there are many studies in which molecular, genetic and physical factors have been investigated in the progression and recurrence of the BC but it has not been fully outlined. All these factors affect the process of metastasis, invasion and resistance to therapy. Generally, major characteristics of malignant cancer cells are defined as self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis, and evading apoptosis. Additionally, tumor metastasis includes detachment, migration, invasion, intravasation, anoikis, evasion, and extravasation steps. In this review, we aimed to summarize the possible genetic, molecular and physical factors that are responsible for the progression and recurrence.

Epithelial-Mesenchymal Transition and Plasticity

Mechanism of the invasion/metastasis includes separation from the epithelial collective, degradation of the surrounding matrix, migration and invasion through the basement membrane, intravasation and survival in the circulation, extravasation at a secondary site, survival as micrometastasis, and, finally, growth into overt metastases (3). Epithelium is an important member of the growth, differentiation, division, apoptosis, tissue integrity and function. Epithelium is organized as sheets of cells attached to an underlying extracellular matrix (ECM) called basement membrane (BM), especially laminin. Epithelial cells are polarized along an apical basal axis and connected to each other by multiple cell-cell adhesive junctions (4). These ensure mechanical integrity of the epithelium and include adherens

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junctions (zonula adherens) and desmosomes (macula adherens). Both of them include a protein named E-cadherin and it is crucial for the current EMT process. MIBC expresses molecular markers of a developmental process known as 'epithelial-mesenchymal transition (EMT)'. On the contrary, mesenchymal cells do not form a layer neither are they attached firmly to a basement membrane. They contact other mesenchymal cells only focally and can migrate easily. EMT process include specification of a cell, disruption of the BM, change in the cell shape, withdrawal from the epithelial sheet and differentiation to a mesenchymal cell (5). Morphological features of a mesenchymal cell include front end-back end polarity, fibroblast-like view, elongate morphology (spindle-shaped), filopodia (front end cell membrane prominences interacting with the surrounding ECM) and invasive motility (6,7). As we know, cellular plasticity is fundamental to embryologic development and plasticity describes differentiation and transformation ability of a cell. EMT process is not cancer but plays an important role in tissue differentiation, organ development, embryogenesis, inflammation, tissue regeneration and wound healing. On the contrary to the EMT, mesenchymal-epithelial transition (MET) is critical for the later stages of metastasis (8,9). MET is a fundamental embryologic process, especially in the nephrogenesis. As we know, trigone region of the bladder and kidney develop from common embryologic origin and several growth factors are critical regulators of this process as wnt/wingless, bone morphogenic protein (BMP) family, fibroblast growth factor (FGF), fibroblast growth factor receptor (FGFR) and proteoglycans (PGs). There is evidence to suggest that the FGF family plays a fundamental role in cellular differentiation and tumor phenotype in bladder carcinoma via MET process (9). Recently, there is also accumulating evidence that EMT/MET plays important roles in cancer progression, invasion and metastasis, resistance to the apoptosis, and refractory responses to chemotherapy. It is characterized by downregulation of the homotypic adhesion molecule, E-cadherin, and proteins involved in cell polarity, with parallel upregulation of fibronectin (FN), vimentin, certain integrins, matrix metalloproteases (MMP), and several transcriptional repressors of E-cadherin expression (Twist, Snail, Slug, Zeb-1, Zeb-2) (10). These transcriptional factors, after binding to the complexes, translocate to the nucleus and transactivate target genes that include oncogenes and tumor suppressors. Vascular endothelial growth factor (VEGF) and FGF-2 are members of the EMT-related genes. Especially, FGFR plays a role in caellular differentiation and epidermal growth factor receptor (EGFR) is correlated with the transition from superficial to invasive BC. EGFR binds two specific ligands: EGF and transforming growth factor-alpha (TGF-α). Members of the TGF-beta/BMP family of cytokines are the best-characterized inducers of EMT, although many inflammatory cytokines (and their transcriptional target, NFκB) and developmental signaling systems (sonic hedgehog, Notch, and Wnt) play central roles in regulating EMT as well. In this process, polarized epithelial cells progressively alter their junctional and polarity complexes to acquire morphological and biochemical characteristics typical of mesenchymal cells (11). Recently, there have been many studies about tumor-initiating cells (cancer stem cells) in the literature. Sonic hedgehog (SHH)-expressing stem cells in basal cells within precursor lesion become tumor-initiating cells (12). Cells in the EMT process exhibit stem cell-like properties. Otherwise, there is no quantitative measure to assess the interplay between EMT and cancer progression (i.e. Spindle Index) (13).

Cadherins (calcium-dependent; types E, N, P) are transmembrane glycoproteins responsible for cell-cell adhesion. The cytoplasmic domain of the cadherins binds catenins (α, β, γ; plakoglobin) which mediate the connection between epithelial E-cadherin and cytoskeletal protein F-actin in adherens junction and desmosome. Interaction forces between two E-cadherin molecules are reported to be 200 nanoNewtons (14). Baumgart et al. (15) have reported that E-cadherin and N-cadherin were associated with the grade and stage of BC. E-cadherin is one of the hallmarks of an epithelial phenotype and N-cadherin is found in fibroblast and muscle cell connections. 'Cadherin switch' from E- to N-cadherin is descriptive for EMT process (15). P-cadherin is localized to the basal cell compartment. Rb tumor suppressor protein regulates E-cadherin expression. Integrin-linked kinase participates in signaling cascades resulting in E-cadherin repression. Alterations associated with the cadherin/catenin complex often feature at the center of EMT related to increased migration and invasion of cells (16). In addition to the N-cadherin, vimentin expression, the second mesenchymal marker, is linked to EMT and displays fibroblast morphology.

According to the recent studies, EMT is strongly associated with aggressive BC behavior, such as recurrence, progression, and metastasis. In the context of these data, increased possibility of the EMT may be a new target in BC treatment strategies. For example, loss of E-cadherin expression is a marker of poor response to the monoclonal antibody cetuximab, which blocks EGFR binding and thereby downregulates BC proliferation (17). In addition, EGFR and miR-200 family members have been found to be predictive of cisplatin-based chemo-responsiveness. EMT reversal may be associated with the responsiveness of high-risk patients with NMIBC to Bacillus-Calmette-Guerin treatment via tumor necrosis-related apoptosis-inducing ligand association. In the future, N-cadherin and Twist targeting therapies (i.e. ADH-1) will be useful for BC treatment.

There is increasing evidence that glicosphingolipids (GSLs) and glycosylation status of proteins play key roles in oncogenesis (18). GSLs and protein glycosylation are therefore expected to play some role in EMT process. The ability of GSLs and gangliosides to interact with various signal transducers, as well as with receptors for growth factors (GFs) or integrins, to define cell adhesion, motility, and growth were well established in many previous studies (19,20,21).

Cytoskeletal Filaments and Extracellular Matrix

The three major cytoskeletal filaments are the microfilaments (MF) (actin; polymerized highly conserved protein), intermediate filaments and microtubules. MF and microtubules (cytoskeleton) play an important role in cellular communication and intracellular transport and signaling processes, cellular movement and motility (cytokinesis), migration, differentiation, membrane organization, cellular growth, cell division, phagocytosis, and molecular transport between the plasma membrane and the nucleus. Increased motile activity, increased rate of cell proliferation and removal of growth inhibiting cell-cell contacts are hallmarks of tumorigenesis. Actin exists either in monomeric (G-actin) or polymeric forms (F-actin). Most filaments also contain a tropomyosin (TM) polymer that runs along the major groove in the microfilament (22). Microtubules are composed of α/β tubulin heterodimers. Intermediate filaments (IFs) are the principal structural determinants within cells. IFs can be divided into five

classes: keratins, neurofilaments, desmin, laminin, and vimentin. IFs are linked to the ECM and extend to the cytoplasmic interior that surrounds the nucleus.

The process of cell motility (CM) can be broken down into four steps; protrusion, adhesion, contraction and retraction. Successfully crossing many of the physiological barriers to tumor cell metastasis (i.e. basement membrane) requires specialized structures, such as invadopodia and podosomes. There exist different modes of cell migration, such as mesenchymal and amoeboid movement. Activation of CM and migration is caused by activation of receptors, turning on the growth cycle. Increased expression of MMPs, breaking cell-cell contacts allows journey of the malignant cancer cells. Most transmembrane proteins (GF receptors, adhesion proteins, ion channels) are either permanently or transiently associated with the submembranous system of actin MF. Redox control of the actin (MF) system in CM and migration is an emerging field of research. In a few studies, MIBC, which activates RhoA/ROCK signaling pathway, has been shown to promote the enhanced contractility of cells using amoeboid migration (23,24).

In this system, transmembrane proteins, linked to the submembranous actin force generator, are responsible for the first level of the CM, and shape and integrity of cells, whose four steps are:

1) Polymerization of actin into filaments,

2) Organization of filaments into ensembles by cross-linking proteins and by adhesions to extracellular structures,

3) Force-generation for large scale movements through interaction between actin filaments and different myosins,

4) Depolymerization of filaments to reform unpolymerized actin for new rounds of polymerization (25).

During this cycle, MF system is executed in response to interactions between the cell and surrounding environment, i.e., GFs, cytokines, other cells or extracellular matrices (local area network). Cell surface protrusions (lamellipodia and filopodia) are built of actin microfilaments, whose assembly takes place primarily at advancing edges of cells, and it is the polymerization of actin that provides the force for their protrusion (26,27). Myosin-dependent processes translocate molecules and particles along lamellipodial and filopodial MF arrangements. For example, integrins are transported towards the tip of filopodia and they become involved in filament growth and establishment of adhesion sites. Stress fibers (MF and myosin II) are used to move the whole cell (28). In tissues, cell-cell interactions engage different transmembrane proteins, e.g., cadherins which dynamically link actin microfilament arrangements in neighboring cells by mechanisms that are crucial in this process (29,30,31,32). Many of the proteins involved in the control of the MF system are products derived from proto-oncogenes. Endo- and exocytosis and formation of the podosomes, invadosomes, filopodia are major actors in this process. Actin filaments are attached to profilin in the cell. On the other hand, TM may be critically involved in the regulation of actin filament formation and function, as reflected in the alterations in TM isoform expression seen as a result of the development of the malignant state of cancers (33,34). GF-stimulated cells rapidly change their levels of TM isoforms in the cytosol, which coincide with actin polymerization, leading to formation of lamellipodia and filopodia. Gelsolin (fragmentation) and cofilin (disassembly) are important molecules in this phosphoinositides- and CM-cycles. In addition to polyphospho-inositides and small GTPases, transient generation of H_2O_2 seems to play important roles in regulating formation and activity of cell edge protrusions, integrin-mediated adhesion and migration (35,36). Cofilin controls crucial aspects of motility and migration of cells. Malignant cells have strongly altered levels of TMs in the cytosol and cytomatrix. High molecular weight and low molecular weight isoforms appear to influence different aspects of the functioning of the MF-system; one class primarily being involved in controlling the motile activity of lamelliopodia and filopodia, and the other controlling the formation of cell adhesions and stress fibers. Profilin, cofilin, TM, gelsolin, α-actinin, and vinculin, belonging to the MF system, are associated with malignant transformation. Cell migration is executed by repeated cycles of protrusion (actin polimerization), matrix adhesions (formation of focal complexes/ focal adhesions in association with actin filaments) and retraction (actomyosin force generation). Integrins appear at the outer edge of cell protrusions and presumably as a result of integrins interacting with the ECM, focal complexes (integrin, talin, paxilin, vinculin, zyxin, tensin) appear at a distance of about 1µm from the advancing edge (37,38). Recently, it has been discovered that stress forces from outside or actomyosin-dependent forces positively influence (mechanosensing) actin polymerization at the focal adhesions (39). Integrins execute cell-matrix as well as cell-cell interactions, whereas adhesion via the cadherin family of adhesion proteins is preferentially intercellular. Cadherin-cadherin interactions link the MF system of adjacent cells. For example, E-cadherin, the protypic member of the cadherin family, regulates cell adhesion in epithelial cells. During embryonic development, down regulation of E-cadherin function initiates a complex program wherein epithelial cells adopt a fibroblast-like phenotype and display tissue invasive activity, a process called EMT. Repression of E-cadherin appears to play a major role in EMT of epithelial-derived cancer types. E-cadherin repression frequently occurs in tandem with activation of the Wnt-signaling cascade (40).

Physical Factors and Reciprocity

Elasticity of BC cells (BCC) are strongly linked to the actin cytoskeleton (spatial 3D organization and density, stress fibers). Elasticity measurements are performed with an atomic force microscope at the cellular level. BCC have Young's moduli (Pascal-Pa) about 2-3 times lower than that of non-malignant cells. The low Young's modulus (higher cellular deformability) seems to occur at an earlier stage of cancer progression and it does not include the metastatic phenotype (41). Its occurrence depends on partial lack and/or depolymerization of the actin filaments. Evaluation of the stiffness can be used as a biomarker of BC. This configuration plays a dominant role in controlling the elasticity of BCC to an external force (i.e. intravesical pressure). MRI elastography will be useful instrument for this purpose (42). Effert and Seifert (43) reported that ultrasutructural analysis of microinvasions in the basal epithelial cells may help to evaluate the invasion capability for BCC by electron microscopy. Moreover, for tissue invasion, cancer cells are able to move out and infiltrate adjacent tissues by degrading enzymes. Cancer invasion can be described as a morphological instability that occurs during tumor growth and results in invasive 'fingering' and

branching. This instability may be driven by any physical or chemical condition. Adhesion molecules, such as cadherins are the determinant factor of the physical environment. In recent researches reported that, in 3D environments, E-cadherin deficiency indeed led to a loss of intercellular adhesion and triggered tumor cell invasion by matrix MMP-2 and MMP-9 driven matrix degradation. Surface tension at the tumor-tissue interface, have been extensively studied in the field of fluid dynamics (44,45,46,47). Promoting tumor cell adhesion and thus increasing the tumor surface tension can induce cellular cohesiveness and decreasing of the invasiveness. Microenvironmental pressure and tumor radius can be determinant of the invasion capability. Reducing the tumor size through and accompanying nonsurgical approaches may provide additional contributions by decreasing the invasion. Reducing the confining mechanical pressure exerted on the tumor can affect therapy results (i.e. adjuvant corticosteroid treatment).

Hyaluronan Regulation and Glycosaminoglycan

The urothelium, the epithelial lining of the bladder, also known as the transitional epithelium, is not just a simple barrier. It is now recognized as a specialized tissue that regulates complex bladder function. The surface of the urothelial umbrella cells carry a thin layer of glycoproteins and PGs, together forming a glycosaminoglycan (GAG) layer which constitutes a hydrophilic mucosal coating and a barrier against solutes or noxious substances in the urine (48,49,50). There is evidence that the implantation and seeding of viable tumor cells influence BC recurrence and endeavoring to prevent early implantation would appear to be a worthwhile therapeutic focus (51). The current clinical approach involves chemotherapy with instillations of cytotoxic agents, and two recent proposals under investigation describe an anti adhesive application and an antiangiogenic strategy (52). Providing a more protective barrier or blistering the GAG layer of the urothelium to prevent implantation of tumor cells is another option with therapeutic potential in recurrent BC (53).

Damage to the urothelial GAG barrier layer is thought to underline the pathologies of several chronic bladder pathologies. Penetration of urinary constituents into the bladder wall causes C-fiber activation, mast cell activation and histamine release. Protecting the urothelium or restoring the GAG layer to prevent the inflammation is the basis for clinical use of intravesical instillation (sodium hyaluronate-chondroitin sulfate). GAG replacement therapy in cancer is being investigated.

GAGs are unbranched polysaccharides composed of repeating disaccharide units of alternating uronic acids and amino sugars. Most GAGs are covalently attached to core proteins to form PGs. Dysregulated expression of GAGs can be associated with angiogenesis, cancer, inflammation, GF signaling, proteolysis of the environment, and cell behavior. Four major classes of GAGs have been identified: heparan sulfate, chondroitin sulfate/dermatan sulfate, keratan sulfate, and hyaluronan (HA). PGs are classified based on the amino acid homology of their protein cores, their location (cell surface, basement membrane, ECM) and their GAG substitution (54,55). Some PGs are substituted with more than one GAG chain type, such as syndecan-1 (heparan sulfate and chondroitin sulfate) and aggrecan (keratan sulfate and chondroitin sulfate), versican, perlecan, decorin, biglycan, glypican, etc. Cancer-related functions of GAG receptors and enzymes are involved in GAG synthesis and modification. GAG and PGs play important roles in multiple cancer-related processes. GAGs and PGs are effective in controlling cell proliferation. Cell surface heparan sulfate PGs serve as coreceptors for several GFs. Chondroitin sulfate PGs have a role as modulators of signal transduction and enhance focal adhesion kinase. Dermatan sulfate PGs decorin modulates EGFR signaling and controlling cell proliferation. The ability of cancer cells to invade into surrounding tissues involves changes in expression of cell surface molecules and the expression of ECM-degradative enzymes. GAGs and PGs are major constituents of the ECM and cell surface PGs mediate cell-matrix interactions. Changes in expression of these molecules reduce cell adhesion and promote cancer cell invasion. Versican inhibits cell adhesion to FN, syndecans acting in concert with integrins, HA signaling through CD44 contribute to increased cancer CM through signaling events that activate the cytoskeleton. Cancer cells also secrete matrix metalloproteinase (MMP), heparanase, hyaluronidases to penetrate the BM and ECM to invade surrounding tissues (56,57,58,59). Metastasis includes cancer cell dissemination into the circulation, adhesive interactions with endothelial cells, and colonization. Heparanase promotes invasion and metastasis by degrading heparan sulfate chains in cell surface and matrix heparan sulfate PGs (60,61,62). Syndecan-1 may regulate the adhesion of cancer cells to blood and lymphatic vessel endothelium or promote the association with different host cells during metastatic seeding. HA activates EMT. For a cancer to grow beyond a diameter of 2 mm, primary tumors and metastases require nutrient support from the vascular system. In this process, crucial molecules are VEGF, FGF, angiopoietins, GAGs, and PGs (56,63,64,65,66,67).

Proteinases contribute to all stages of diseases, especially cancer development and progression. ECM is a highly dynamic and functional network. Major ECM components are PGs, as well as fibrillar proteins, such as collagens and elastin, and other glycoproteins. In cancer, PG expression is often altered in the stroma and this might contribute to disease progression that is occurred via matrix proteinase. Extracellular proteases are actively involved in tumor progression and metastasis by degrading the majority of ECM macromolecules. Proteinase enzyme family catalyzes the hydrolytic breakdown of proteins into peptides or amino acids at their terminal ends (exopeptidases) or inside the peptide chain (peptidases). At least 569 proteinases are defined according to the MEROPS database and distributed intra and extracellularly which are classified based on the chemical moiety that participates in the hydrolysis (aspartic, cysteine, threonine, serine and metalloproteinases) (68). A single PG would be expected to interact and modulate more than ten proteases. Proteinases contribute to all stages of tumor progression, including tumor growth and survival, angiogenesis, cell invasion, cell adhesion, migration, EMT, and immune surveillance, and that they are produced not only by the tumor cells themselves, but also by the tumor microenvironment (69,70). Metalloproteinases and cathepsins are among the major families of proteinases implicated in cancer progression. Metzincin family of MMPs (including methionine residue and zinc) is comprised of matrix MMPs, a disintegrin and MMPs (ADAMs), ADAMs with thrombospondin motifs (ADAMTSs), bacterial serralysins, and proteases such as the astacins (including the meprins) (71,72). The current MMPs are classified into six groups as collagenases, gelatinases, stromelysins, matrilysins, membrane type MMPs, and other MMPs (73). MMPs are the main group of regulating proteinases in ECM. MMPs are responsible for the turnover and

degradation of almost all ECM components (collagen, laminin, FN), non-ECM cell regulators, integrin, kinases, chemokines and cytokines (Table 1) (74,75).

Heparin blocks the effect of MIBC exosomes on cell migration and invasion that uptake occurs through a heparin sulfate proteoglycan (HSPG)-dependent mechanism (76). HSPGs often act as a co-receptors for various integrin receptors and integrin receptors are well-established mediators of cell-matrix interactions (including tumor migration and invasion) (77,78,79,80,81). However, this mechanism remains to be elucidated.

Angiogenesis

Angiogenesis is fundamental to tumor growth, invasion and metastasis. Hypoxia plays a key role in tumor progression by modulating gene regulation and expression, such as hypoxia-inducible factor 1 (HIF-1). Another aspect of the tumor microenvironment that has a role in tumor metastasis is inflammation. Dysregulation of the normal wound healing processes in cancer can result in an influx of angiogenic cytokines from nearby immune cells contributing to metastasis.

The VEGF plays pivotal roles in tumor angiogenesis. Blockage of the VEGF signaling as a therapeutic target includes antibodies, aptamers, peptides and small molecules. For tumor clones to grow beyond 100-200 µm, they have to recruit new blood vessels by angiogenesis (82,83,84). Tumor angiogenesis also involves a complex interplay between the tumor and surrounding or supportive cells, including vascular endothelial cells, pericytes, smooth muscle cells, fibroblasts and tumor-associated macrophages (85). In response to hypoxia, tumor tissues produce angiogenic GFs such as VEGF, fibroblast GFs (FGFs), and platelet-derived endothelial cell growth factor. These angiogenic growth factors bind to their corresponding specific receptors located on the endothelial cells of preexisting blood vessels; various signal transduction pathways are activated to promote the activation of endothelial cells (86,87,88). Subsequently, the original vessels undergo characteristic morphological changes, including enlargement of the diameter, BM degradation, a thinned endothelial cell lining, increased endothelial number, decreased number of pericytes, and detachment of pericytes. At the sprouting tips of growing vessels, endothelial cells secrete MMPs to facilitate the degradation of extracellular tumor matrix and cell invasion (89). Cell surface adhesion molecules, such as integrins also play an important role in endothelial cell migration and in contact with the extracellular tumor matrix, facilitating cell survival (90,91). Next, a lumen within an endothelial cell tubule has to be formed, which requires interactions between the ECM and cell-associated surface proteins, among them are galectin-2, PECAM-1, VE-cadherin (92). A fast growing tumor almost always creates a hypoxic environment due to several interconnected reasons

Table 1. Extracellular molecules involved in cancer invasion

Category	Molecule	Type	Molecules co-involved in cancer cell invasion
	HA	Glycosaminoglycan	CD44
ECM molecules	FN	Glycoprotein	eHSP90, HSP90, MMP-9, FAK/PI3K/AKT/ERK/ NF-κB, PEDF
	Sibling	Small Integrin-Binding Ligand, N-linked Glycoprotein	Pro-MMPs, MMP-2, MMP-9, MMP-3, αvβ3 integrin, FAK/MEK/ERK/NF-κb CD44v6
ECM receptors	Integrins	Cell surface receptors	FN, MMP-9, MMP-2, FAK/ILK/ERK/PI3K/ NF-κb, EGFR, osteopontin
	CD44	Cell surface receptors	HA, osteopontin
Growth factors	TGF-β	Growth factors	TBRI, TBRII, Erk, Ras
	Heregulin	EGF-like growth and differentiation factor	ErbB3, ErbB4, PAK-1, AMF
Growth factor receptors	EGFR	Cell surface receptor	TGF-α, Grb-2, Ras/Raf/MEK/MAPK
	HER-2	Cell surface co-receptor	HER-3, eHSP90, MAPK, PI3K/AKT
	IGF-R	Cell surface receptor	IGFs, IRS-2, PI3K/AKT, Ras/Raf/MAPK
Matrix metallo proteinase	MMP-9	Zinc endopeptidase	eHSP90, HASP90, Rab40b, VAMP4, gelatin type IV, collagen, VEGF, bFGF
	MMP-2	Zinc endopeptidase	Gelatine, type IV collagen, eHSP90, HASP90, Rab40b, VAMP4, VEGF, bFGF
	CD-10	Zinc dependent metalloproteinase	Twist 1
Chaperones	eHSP90	Chaperon	Cdc37, FN, HER-2, EGFR, pro-MMP2, pro-MMP9
	eCdc37	Co-chaperon	HSP90, eHSP90, HER2, EGFR, Raf-1, CDK4, EGFRvIII, PJCS-AK
LRP-1	LRP-1	Low density lipoprotein receptor	Nexin-1 (PN-1), Erk pathway, MMP-9, eHSP90, EphA2, AKT1, AKT2

ECM: Extracellular matrix, LRP-1: Low density lipoprotein receptor-related protein 1, EGFR: Epidermal growth factor receptor, IGF-R: Insulin-like growth factor receptor, MMP: Matrix metalloproteases, VEGF: Vascular endothelial growth factor, TGF-α: Transforming growth factor-alpha, TGF-β: Transforming growth factor-beta, FN: Fibronectin, HA: Hyaluronan

including unsynchronized growth rates of tumor and endothelial cells, disorganized vascular architecture, sluggish blood flow and high interstitial fluid pressure (IFP) (93,94). Hypoxia leads to increased levels of HIF-1 alpha which increases VEGF expression. High level of VEGF could further increase vascular disorganization, permeability and IFP, leading to severe hypoxia in turn (95).

Tumor associated endothelial cells can acquire cytogenetic abnormalities while in the tumor microenvironment (intratumor ecosystem). Altered expression of VEGF has been observed in urothelial carcinoma of the bladder (UCB) cells (96). Elevated levels of VEGF expression have also been detected in urine samples from UCB patients and correlated with disease recurrence and progression (97). High level of VEGF expression in tumors and in serum samples from patients with UCB also predicted poorer prognosis and increased frequency of disease recurrence (98). Altered VEGF expression has been found to be associated with advanced pathological stage and lymph node metastasis (99). It is suggested that VEGF, in combination with other angiogenic factors such as angiogenin and MMPs, may serve as a biomarker for the diagnosis and prognosis of patients with UCB.

Conclusion

Each BC in various stages and grades is considered as a different disease. NMIBC is a well curable cancer, potentially it can display recurrence or progression. Moreover, 30% to 50% of these patients have recurrences after transurethral resection of the primary tumor, and 10% to 20% progress to MIBC. We do not know which factors are exactly responsible for recurrence and progression in BC. In this review, we summarized possible molecular factors in this process. In the future, there is need for more experimental studies on these processes for prognostic evaluation and treatment options of BC.

Ethics

Peer-review: Internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Fehmi Narter, Concept: Fehmi Narter, Design: Fehmi Narter, Data Collection or Processing: Fehmi Narter, Analysis or Interpretation: Fehmi Narter, Literature Search: Fehmi Narter, Kubilay Sabuncu, Writing: Fehmi Narter, Kubilay Sabuncu.

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