Course : PG Pathshala- Biophysics

Paper 11 : Cellular and Molecular Biophysics

Module 16 : Cell motility & Cancer

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Introduction

Metastasis is the leading cause of mortality in cancer patients. Despite many years of cancer research, scientists still do not have a definitive explanation as to how cancer cells migrate out of the primary tumor and invade into surrounding and distant organs. These cancer cells enter blood circulation or lymphatic circulation to reach a certain target organ and then leave the circulation to extravasate and initiate a metastatic outgrowth away from primary tumor. A number of molecular pathways published in literature has contributed to our current understanding of migratory and invasive capabilities of cancer cells. Migratory cancer cells undergo dramatic molecular and cellular changes to become motile and initiate metastatic cascade. Actin cytoskeleton remodelling of cell-cell adhesion and cell-matrix adhesions during cell migration and invasion is one of the critical factors for onset and progression of metastasis. In this module, we summarise the various concepts on the signalling pathways and molecular mechanisms underlying cancer cell motility.

The various objectives of this module are the following:

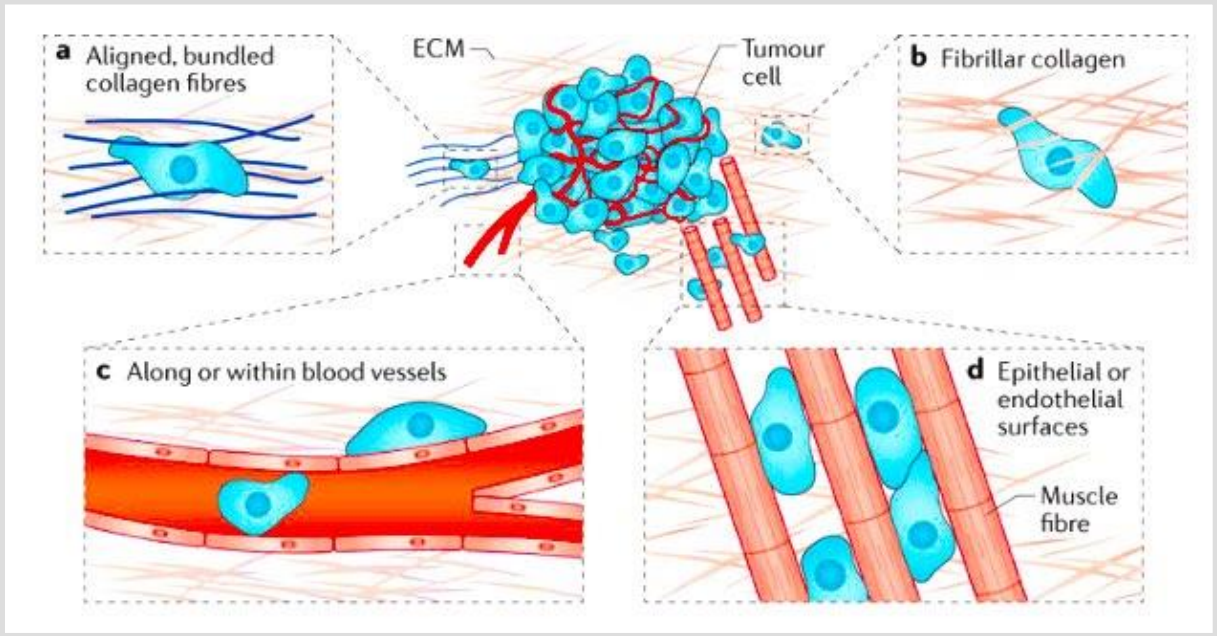
Objectives

- **Initiation of cancer cell motility**
- **The Cadherin Switch in cancer cell migration**
- **Modes and dynamics of cancer cell migration**
- **Role of Microenvironment in cancer cell motility**
- **Conclusions & Perspective**

1. Initiation of cancer cell motility

The first step in metastasis is migration of cancer cell away from the primary tumor, a process called tumor invasion. To leave the primary tumor, cancer cell has to loose its cell-cell contacts followed by a modification in its cell-matrix adhesions to facilitate migration through the extracellular matrix (ECM) by either degrading the matrix using secreted enzymes to create their own migration tracks or by following 'leader' cancer cells or cancer associated fibroblasts that open up migration paths.

Using intravital microscopy it has been shown that cell migration tracks occur naturally in healthy tissue. For example collagen fibres in the interstitial spaces, along blood vessels, between muscle and nerve fibres etc. These tracks offer paths of 'least resistance' for cancer



Anatomic tissue structures guiding cancer cell migration. a | Alignment and bundling of collagen fibres at the tumour periphery provide cues for directed migration. b | Cells may also migrate through unbundled extracellular matrices (ECMs), such as fibrillar collagen, which present pore-like migration spaces. c | Microtracks also occur both intravascularly and perivascularly. d | Cells can also migrate between epithelial or endothelial surfaces, such as those found between muscle and nerve fibres. *(Paul et al Nat Rev Cancer. 2017)*

cell migration.

There is *in vivo* experimental evidence from both human and mouse cancer cell migration studies that cancer cells migrate along the paths of collagen fibres in primary tumor. Perivascular spaces and white matter tracks in brain have been shown to work as 'highways' for glioma cell migration. It has also been observed that melanoma cells use the outer surface of blood vessels as guidance structures for tumor cell migration and progression.

Cancer cell migration along topographical features is non-destructive and does not require tumor microenvironmental remodelling by proteinases. This plasticity in cancer cell migration could help explain the poor *in vivo* performance of matrix metalloproteinase (MPPs) inhibitors for anti- metastasis therapy in cancer.

In case of epithelial cancers, differentiated epithelial tumor cells lose their epithelial morphology and migrate to distant site to form secondary tumors. This temporary and reversible phenomenon is called as epithelial-to-mesenchymal transition (EMT). EMT initiates or augments Rac-dependent mesenchymal cancer cell migration. Invading cells often display up regulation of Vimentin, down regulation of E-cadherin and lose the apical-to-basal polarity. Despite these changes, human cancer migratory cells do not typically show spindle-shaped mesenchymal morphology. Furthermore, the distant metastasis recapitulates the epithelial morphology of primary tumor, which suggests that upon reaching the secondary metastatic niche, the migratory cancer cells that had undergone EMT, reverse these changes by another mesenchymal-to-epithelial transition (MET).

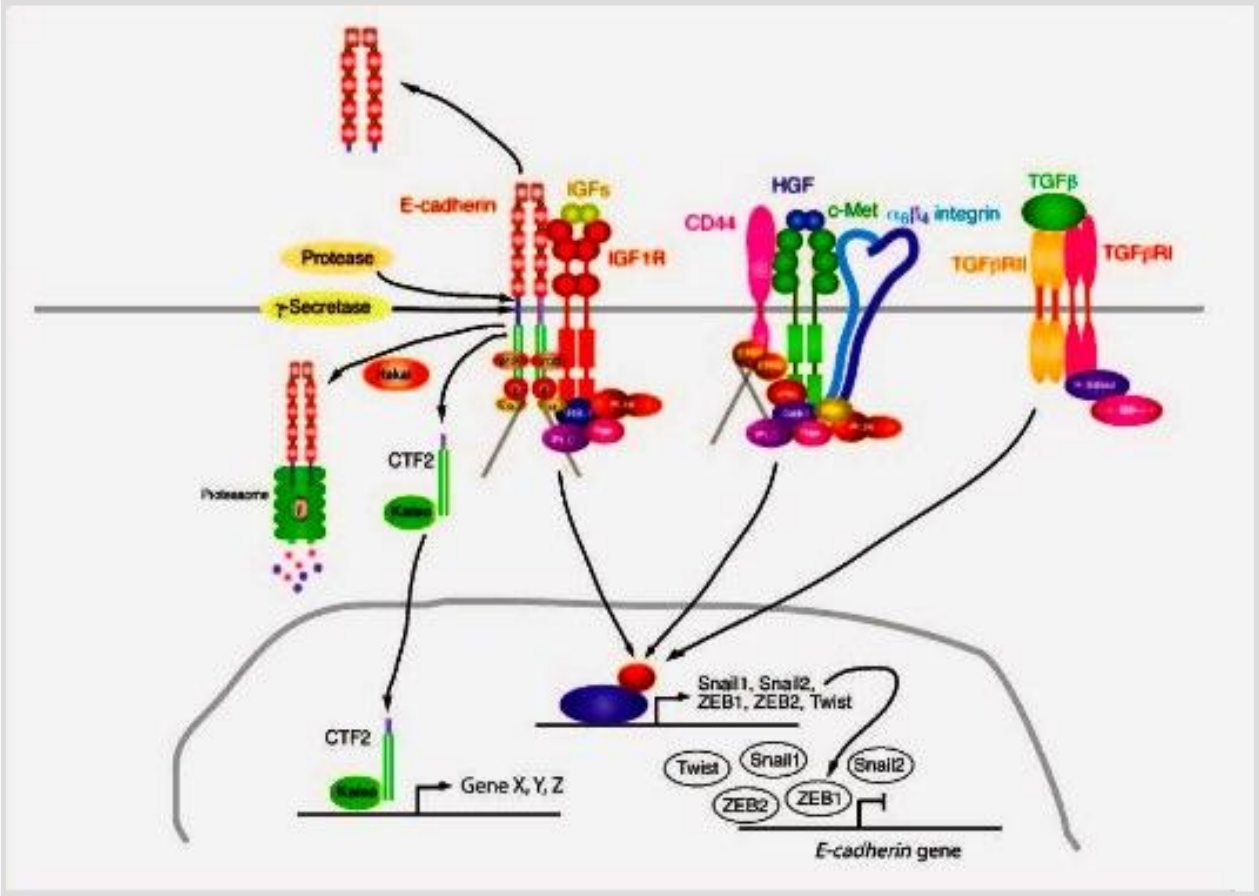
2. The Cadherin Switch in cancer cell migration

One of the important EMT markers is loss of epithelial cell-cell adhesion molecule E-cadherin. Concomitantly, the expression of mesenchymal cell-cell adhesion molecule N-cadherin increases, a process known as 'Cadherin Switch'.

2.1 Loss of E-cadherin

It has been observed that loss of E-cadherin function due to germline/somatic gene mutation, chromosomal aberration, transcriptional repression or DNA hypermethylation of E-cadherin (*cdh1*) gene is sufficient to induce cancer cell migration and invasion and tumor metastasis *in vitro* and *in vivo.* Loss of E-cadherin can be due to molecular alterations caused by a large number of growth factors and their signal transduction pathways including transforming growth factor β (TGF-β), insulin like growth factor (IGF), epidermal growth factor (EGF),

fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and Notch signalling. In addition, hypoxia (frequently existing in tumor microenvironment) induces expression of c-Met, HGF and CXCR4, a receptor for chemokine CXCL12 which induces tumor progression and metastasis. These signals also activate one or more transcriptional repressors of E-cadherin gene expression such as Twist, Snail, Slug, ZEB1, ZEB2, E47 which bind to the E-cadherin gene promoter and lead to epigenetic silencing of this gene. It has been seen that TGF-β induces EMT in mammary gland cells and methylation of E-cadherin promoter region.

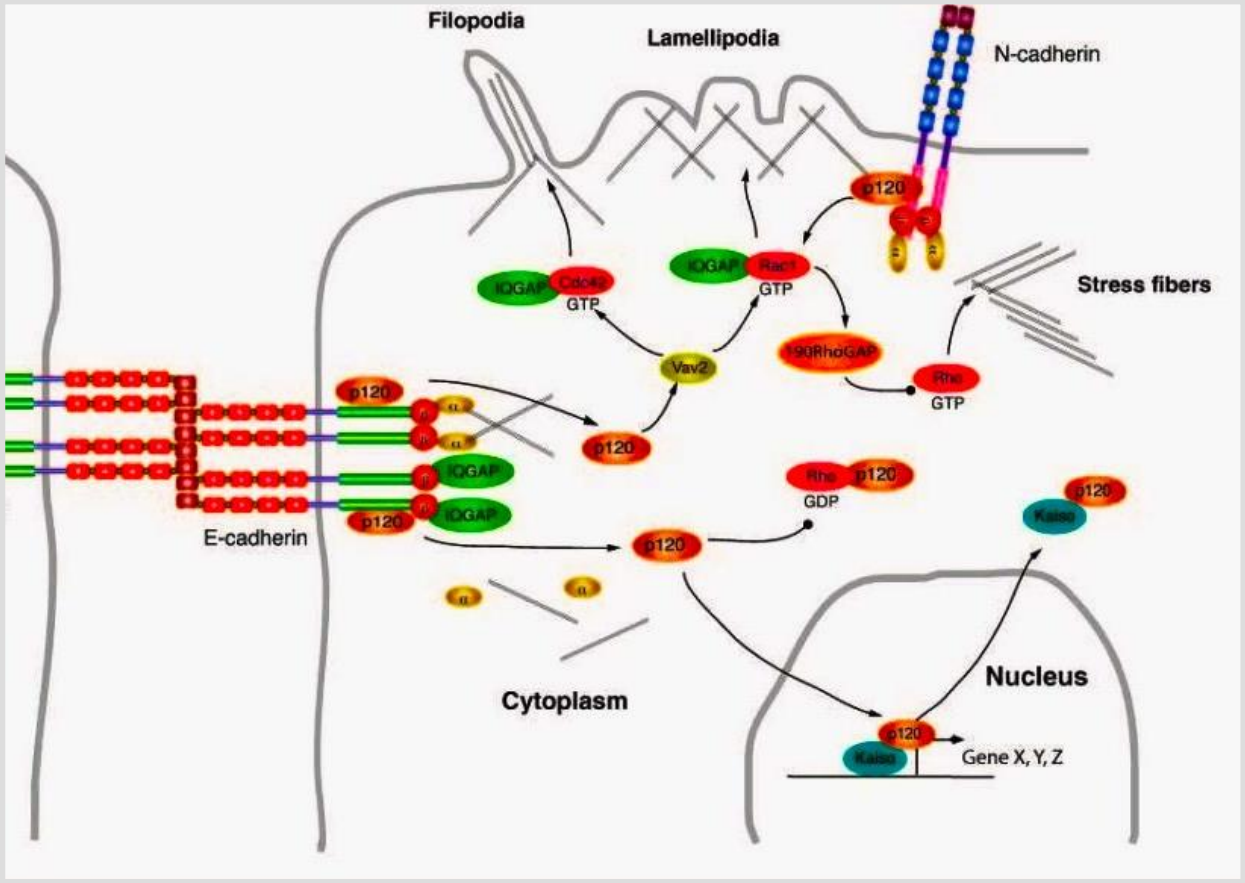


Schematic representation of the signalling pathways leading to the upregulation of transcriptional repressors and the repression of E-cadherin gene expression. (*Yilmaz et al, Mol Cancer Res. 2010*)

Likewise, withdrawal of TGF-β promotes reversal of EMT and re-expression of E-cadherin.

Loss of E-cadherin leads to disruption of cell-cell adherens and tight junctions and loss of cell polarity. Proteins like β-catenin play a critical role in E-cadherin mediated cell adhesion complex. Upon loss of E-Cadherin along with non-functional tumor suppressor APC gene, β-catenin accumulates in the cytoplasm and translocates to nucleus to modulate expression of genes for cancer cell migration and proliferation. An actin-bundling protein, fascia, one of the

target genes of β-catenin helps in filopodium formation and cancer cell invasion. Similarly, upon loss of E-cadherin, p-120 catenin also detaches from cell-adhesion complex and accumulates in cytosol where it represses RhoA and activates Rac and Cdc42. These GTPases are key regulators of actin assembly and regulate migratory membrane protrusions like



lamellipodium and filopodia.

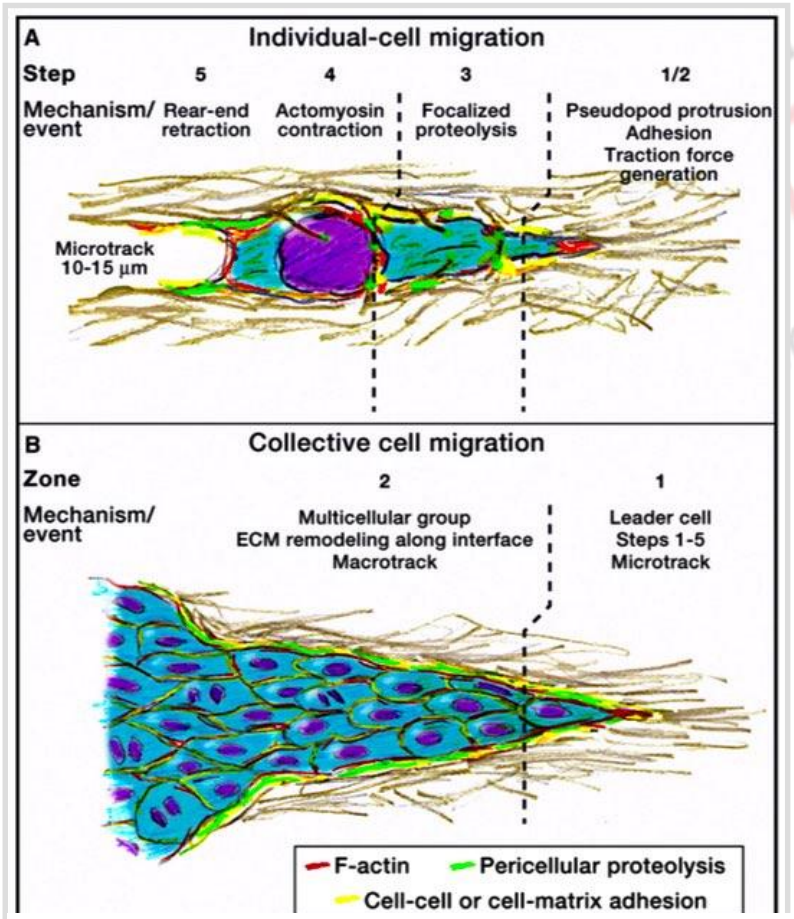
2.2 Gain of N-cadherin

During the cadherin switch, upon loss of E-cadherin function, the transcriptional repressor of E-cadherin, Twist increases the expression of N-cadherin. Also known as mesenchymal cadherin, N-cadherin drastically changes the adhesive properties of cancer cells. With the loss of adherens and cell-cell junctions, they lose their affinity for epithelial neighbours and gain contact with stromal cells. The mechanical engagement of N-cadherin induces actin polymerisation and interacts with various growth factors to modulate signal transduction and cancer cell motility. N-cadherin binds to both β-catenin and platelet-derived growth factor receptor (PDGFR) to induce actin reorganisation, cell proliferation and migration. N-cadherin interacts with fibroblast growth factor receptors to increase cell motility, MMP secretion and

invasiveness just like the neural cell adhesion molecule (NCAM). NCAM is one of the first genes unregulated during EMT and is required for cell adhesion, migration and invasion in many cancers.

3. Modes and dynamics of cancer cell migration

Cell migration is a dynamic process in which the cell changes shape, produces morphological asymmetry, and then translocates the cell body. Cancer cells use similar mechanisms for changing shape, generating force, and remodelling ECM as normal cells, but lack physiological 'stop signal' achieved by cell-cell contact.



(A) Single-cell migration involves five molecular steps that change the cell shape, position, and the microenvironment (B) Collectively migrating cells with 'leader cell' at the front of the migrating group, and 'follower cells' behind.
Friedl et al. Cell 2016

In vivo, cancer cells have been observed to migrate individually, as single cells, as loosely attached strands or chords of tumor cells or as well organised, collective masses with directional migration. The underlying mechanism in all types of cancer cell migration is the dramatic reorganisation of the actin cytoskeleton forming membrane protrusions like lamellipodia, filopodia and invadopodia, coupled with cell surface receptor engagement to the microenvironment through which it migrates.

Various types of cell migration can be observed in different degrees and combinations, but, in general, cancer cells can migrate individually or collectively as multicellular groups. For example, colorectal cancer

cells that have lost E-cadherin disseminate as single, migrating cells. Whereas, squamous cell carcinomas invade as collective cell migration type.

3.1 Single-cell migration

It is characterised by no cell-cell interaction during migration and there is no correlation between the migration pattern between the cell and its neighbours. Cells that migrate singly can show different phenotypes like amoeboid or mesenchymal.

3.1.1 The origin of **amoeboid type** cell migration tumors is often hematopoietic or neuro-ectodermal, including leukemias, lymphomas, and small cell lung carcinoma. In amoeboid type solitary cell migration, cells migrate with low adhesion force or high actomyosin-mediated contractility and adopt rounded shapes. Amoeboid type motility can come in many variants as follows:

a) Cells with short, thin protrusions, devoid of blebs and rapidly changing morphology. They move with high speeds (0.4-5 μm/min).

b) Slower cells with blebbing morphology and disorganised movements.

c) Cells with short protrusions and proteolytic activity moving with low speeds (~0.1μm/min).

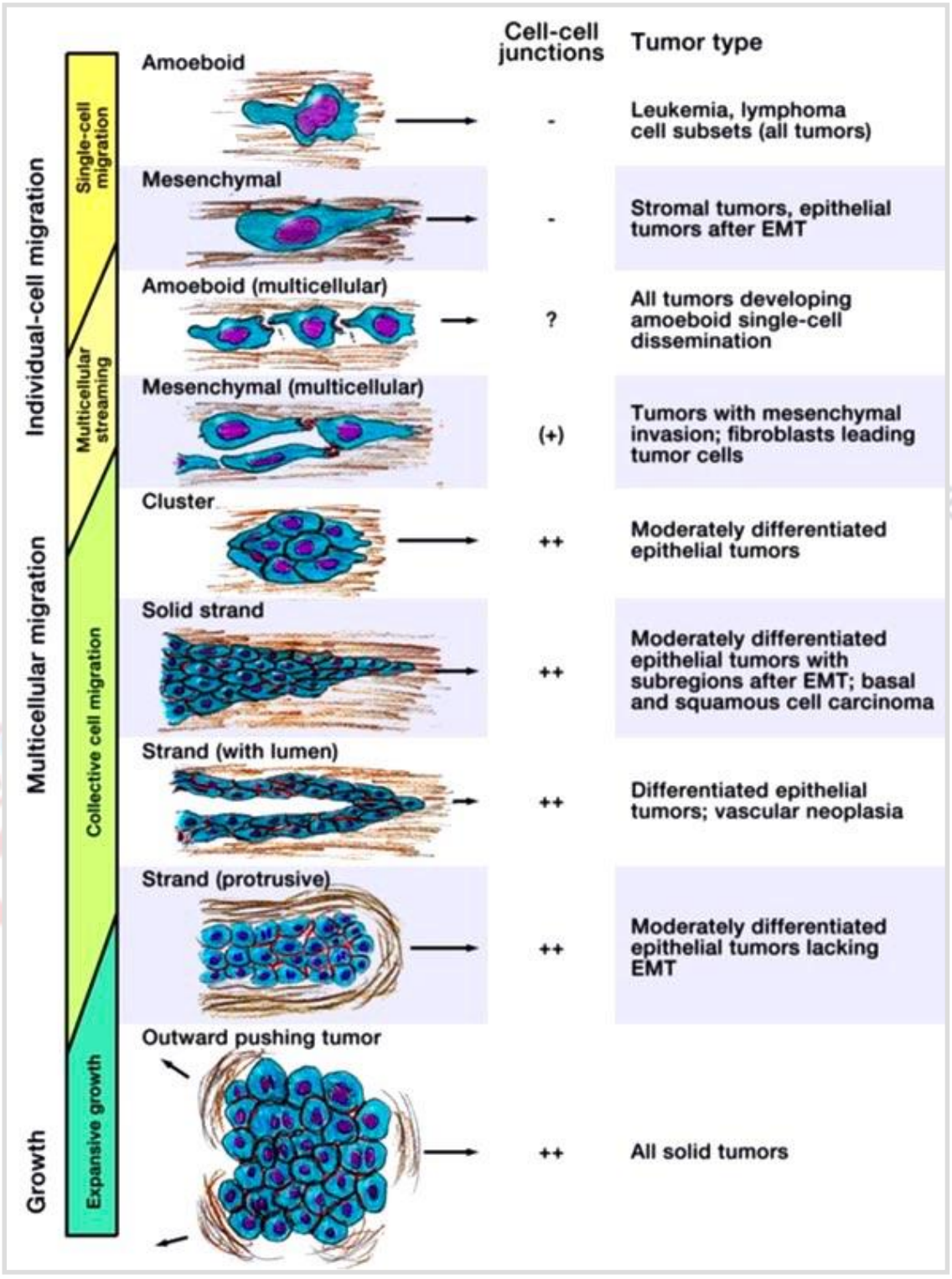
3.1.2 The other solitary migrating type have **mesenchymal** phenotype with spindle shaped cell body and longer protrusions. It has been observed that these protrusions advance rapidly (~0.4 μm/min) but the cell rear moves slowly, resulting in slow net translocation (~0.2 μm/min). Mesenchymally migrating solitary tumor cells originate from tumors of the connective tissue, such as soft tissue sarcomas. They can also originate from other tumors after cellular dedifferentiation and loss of cell-cell interactions.

3.2 Multicellular streaming

When loose, non adherent cells move one after other, in a single file within the tissue, it is referred to as ‘multicellular streaming’. This occurs mainly when individual cells are attracted by chemotactic signals in peripheral connective tissue. Multicellular streaming is seen as

swarm-like tissue infiltration of many tumor cells in haematological malignancies and solid tumors. Cells in streams have longer and straighter paths compared to singly migrating cells and have speeds of 1-2 μm/min. These cells can display amoeboid or mesenchymal phenotype.

Modes of Cell Movement Implicated in Cancer Invasion and Metastasis (*Friedl et al, Cell 2011)*



3.2 Collective migration

Collective migration consists of groups of cells which retain cell-cell adhesion and display high correction of directionality between neighbouring cells. Collectively migrating cells form two major zones: zone A, in which a single 'leader cell' or several leader cells, generate a proteolytic microtrack at the front of the collective group, and zone B, in which the subsequent cells called 'follower cells' then widen this microtrack to form a larger macrotrack. This is the slowest cancer cell migration mode (0.01-0.05 μm/min), but faster collective migration have also been observed (0.2-1μm/min). This type of migration is seen in developmental morphogenesis and in most epithelial and mesenchymal tumor invasion.

4. Role of Microenvironment in cancer cell motility

The molecular and physical characteristics of the microenvironment strongly contribute to cancer cell adhesion, migration and invasion. The most abundant and important component of connective tissue is collagen type I. In healthy tissues, stromal collagen fibres typically appear curly and anisotropic. During early cancer progression, the amount of collagen in stroma increases and collagen fibres straighten out. These collagen fibres bundle and orient perpendicularly to basement membrane to provide tracks for cancer cells to migrate away from the primary tumor.

This reorganisation of stroma is mediated by stromal cells known as cancer associated fibroblasts (CAFs) by secreting ECM and enzymes that covalently link the collagen fibrils. Due to this, the cancer tissue becomes stiffer, while cancer cells themselves appear to become softer. Cancer tissue is two to ten times stiffer than normal tissue (eg. Breast cancer) and this stiffness correlates with increased risk of metastasis and poor prognosis. Along with pro-tumoral collagen, ECM molecules, including collagens, laminins, fibronectin, and elastin; cancer cell surface molecules including cadherins, CAMs, and proteoglycans; and promigratory factors, like chemotactic (soluble factors) and haptotactic gradients (ECM-bound factors) direct tumor cell metastasis and cancer progression.

Integrins also mediate the migration of cancer cells along structural components of basement membranes by engaging with collagen type IV, laminins, fibrillin, perlecan, and versican. Gene expression profiling and proteomics have revealed abundant sets of soluble factors and ECM proteins upregulated in the microenvironment of tumors, indicative of complex signalling pathways induced in both tumor and stromal cells to support cancer cell migration.

In both tumor and stromal cells, multiple protease systems are upregulated including MMPs, ADAMs, cathepsins, and other serine proteases. These proteases contribute to tumor invasion and progression through different mechanisms. Cell surface proteases, MMPs and ADAMs, cause contact-dependent proteolysis of structural proteins, including fibrillar and nonfibrillar collagens, fibronectin, and laminins, as well as tenascin and glypican. Proteolytic ECM degradation generates biologically active epitopes of ECM components with adhesion and migration promoting effects and it remodels stromal collagen tissue to form gaps and tracks for cancer cell migration. These proteases enzymatically activate other proteases and cell surface receptors, including adhesion and growth factor receptors on both tumor and stromal cells and regulate the repertoire of available extracellular growth factors by enzymatic activation, inactivation, or degradation

Extracellular chemokines, cytokines, and growth factors released by tumor cells themselves or activated stromal cells engage intracellular pro-migation signaling pathways in tumor cells. Migration-promoting signals induced by chemokines like CXCL12, CXCL10, CCL21, or CCL25 and their receptors CXCR4, CXCR3, and CCR9 are mainly mediated by JAK/PI3K/JNK, PI3K, Src-family kinase Syk, and the small GTPases Rac1, RhoA, and Rap1.

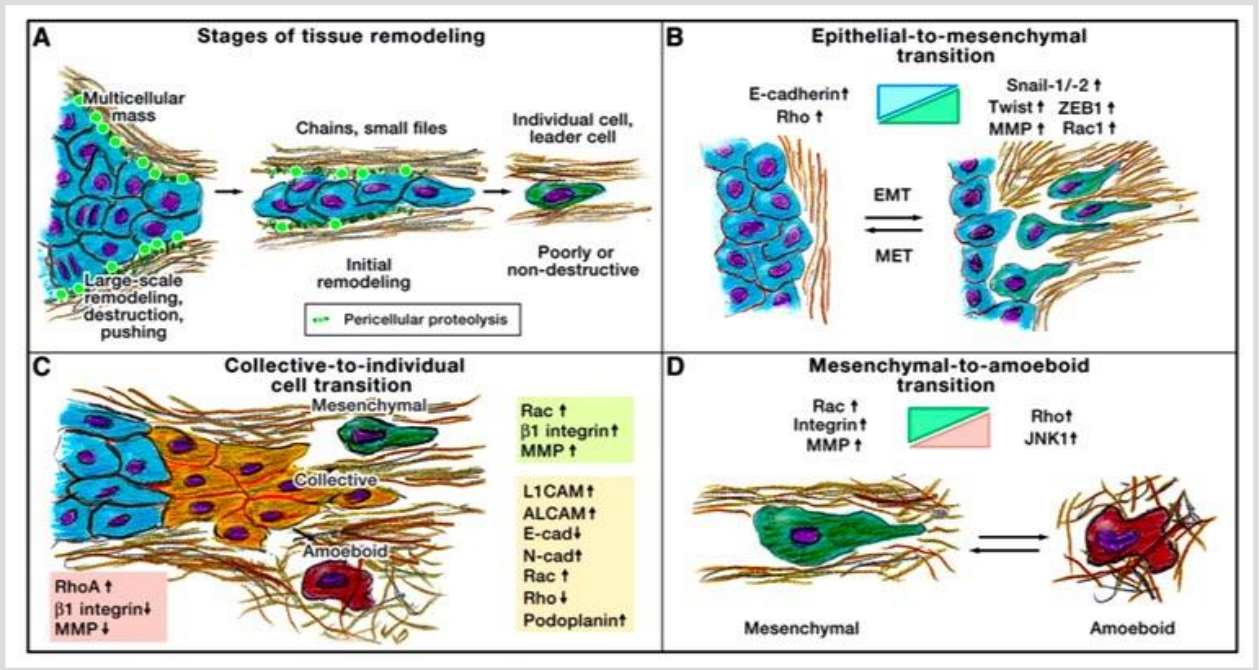
Migration modes are also influenced by tumor microenvironment. A more aligned and organised microenvironment promotes collective migration, such as melanoma cells in dermis that migrate along linear tracks of muscle fibres and nerves. In contrast, cancer cells in fatty connective tissue migrate slowly in multicellular groups lead by several leader cells.

Thus, the diverse and multifactorial involvement of tumor microenvironment in regulating cancer cell migration cannot be over emphasised. Specific alignment of collagen fibres observed using intravital imaging has been termed as ‘tumor associated collagen signature’ (TACS) and has been identified as an independent prognosis factor in breast cancer metastasis. Similarly a diagnostic cellular arrangement of a cancer cell, a macrophage and an endothelial cell, termed ‘tumor microenvironment of metastasis’ (TMEM) is suggested to serve as an independent indicator for metastasis development. These studies allow for development of targeted anti-metastasis therapies in cancer directed towards the microenvironment rather than only tumor cells.

5. Conclusions & Perspective

Cancer cell migration and metastasis is the result of reciprocal crosstalk between tumor cells and the stroma which includes stromal cells together with ECM and released factors. The choice of a wide repertoire of migration modes during metastasis, makes it difficult to predict whether modulation of a given signalling pathway will actually inhibit tumor metastasis in the diverse microenvironments as found *in vivo*.

In human cancers, tumor and stroma evolve together over months and years. Appropriate experimental systems recapitulating the tumor and stromal interactions *in vivo* have to be devised to study the mechanisms of cancer cell migration. Stromal alterations include cell-derived physicochemical changes of the microenvironment, such as deposited ECM components, ECM degradation and remodeling, change of ECM stiffness and porosity, and released cytokines and growth factors. Cancer cell phenotype and function, contributing to migration consists of: changes in the activation, migration, and differentiation state of the cell; metabolic switches; and epigenetic alterations that may further prompt secondary genomic instability. Consequently, with each cycle of interactive engagement with the stroma, the cancer cell state evolves from its origin state, leading to progression of the tumor and its



Plasticity of Cell-Matrix Interaction, Invasion, and Tissue Remodeling (A) Migrating cells transition from an initial nondestructive dissemination to migration that involves small- and large-scale tissue remodeling. (B) Epithelial-to-mesenchymal transition of a stable epithelium after downregulation of cell-cell junctions and facilitated single-cell detachment. Invasion programs display plasticity, including transition from collective cell migration to individual cell migration (C) and mesenchymal to amoeboid transition (D). *Friedl et al. Cell 2011*

metastasis

Future research in cancer metastasis should entail detailed characterisation of both the extracellular microenvironment (including stiffness, adhesion properties, chemotactic signalling, migration tracks and spaces) and cancer cell intrinsic mechanisms (including type

of actin organisation, direction of traction forces and cell surface cues) to elucidate the escape mechanisms cancer cells use to migrate to distant organs.

Summary:

- To leave the primary tumor, cancer cell has to loose its cell-cell contacts (tumor invasion) followed by formation cell-matrix adhesions to facilitate migration through the extracellular matrix (ECM).
- Due to cross-talk between tumor cells and stromal cells, the stroma becomes reactive. Reactive stroma is characterized by an increased presence of immune cells and fibroblasts, which can help to deposit ECM and reorganize the stromal network (mostly made of collagen-I).
- Stromal network fibers are initially loosely organized and appear 'curly' and later increase in density and stiffness. At late stages, collagen bundles form 'tracks' perpendicular to the BM.
- Invading cells often display characteristic Epithelial to Mesenchymal Transition (EMT) markers, such as down- regulation of E-cadherin and upregulation of Vimentin, and lose some epithelial characteristics, such as apical- basal polarity.
- Loss of epithelial cell-cell adhesion molecule E-cadherin and concomitant expression of mesenchymal cell-cell adhesion molecule N-cadherin is known as 'Cadherin Switch' which initiates cancer cell migration and cancer progression.
- *In vivo*, cancer cells have been observed to migrate individually, as single cells, as loosely attached strands or chords of tumor cells or as well organised, collective masses with directional migration.
- Cells that migrate singly can show different phenotypes like amoeboid or mesenchymal.
- When loose, non adherent cells move one after other, following a chemotactic signal within the tissue, it is referred to as 'multicellular streaming'.

- Collective migration consists of groups of cells which retain cell-cell adhesion and display high correction of directionality between neighbouring cells. It consists of a ‘leader cell’ at the front of the migrating group, and ‘follower cells’ behind.

- The reorganisation of stroma is mediated by stromal cells known as cancer associated fibroblasts (CAFs) by secreting ECM and enzymes that covalently link the collagen fibrils which contributes to tumor tissue stiffness.

- Stromal alterations include cell-derived physicochemical changes of the microenvironment, such as deposited ECM components, ECM degradation and remodeling, change of ECM stiffness and porosity, and released cytokines and growth factors help in cancer cell metastasis.

- Targeting the migratory spread of cancer cells requires identification and understanding of the full repertoire of cancer cell migration modes and mechanisms including both cell intrinsic and microenvironmental factors.