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Review

# Physical break-down of the classical view on cancer cell invasion and metastasis

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ABSTRACT

Eight classical hallmarks of cancer have been proposed and are well-defined by using biochemical or molecular genetic methods, but are not yet precisely defined by cellular biophysical processes. To define the malignant transformation of neoplasms and finally reveal the functional pathway, which enables cancer cells to promote cancer progression, these classical hallmarks of cancer require the inclusion of specific biomechanical properties of cancer cells and their microenvironment such as the extracellular matrix and embedded cells such as fibroblasts, macrophages or endothelial cells. Nonetheless a main novel ninth hallmark of cancer is still elusive in classical tumor biological reviews, which is the aspect of physics in cancer disease by the natural selection of an aggressive (highly invasive) subtype of cancer cells. The physical aspects can be analyzed by using state-of-the-art biophysical methods. Thus, this review will present current cancer research in a different light and will focus on novel physical methods to investigate the aggressiveness of cancer cells from a biophysicist's point of view. This may lead to novel insights into cancer disease and will overcome classical views on cancer. In addition, this review will discuss how physics of cancer can help to reveal whether cancer cells will invade connective tissue and metastasize. In particular, this review will point out how physics can improve, break-down or support classical approaches to examine tumor growth even across primary tumor boundaries, the invasion of single or collective cancer cells, transendothelial migration of cancer cells and metastasis in targeted organs. Finally, this review will show how physical measurements can be integrated into classical tumor biological analysis approaches. The insights into physical interactions between cancer cells, the primary tumor and the microenvironment may help to solve some "old" questions in cancer disease progression and may finally lead to novel approaches for development and improvement of cancer diagnostics and therapies.



## Introduction

During the last three decades many aspects of classical tumor biology research have been investigated and hence, eight hallmarks such as sustaining proliferative signaling, evading growth suppressors, avoiding immune destruction, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis, resisting cell death and deregulating cellular energetics have been proposed (Hanahan and Weinberg, 2000, 2011). Among the molecules important for cancer cell motility and invasion are E-cadherin-Notch signaling, integrin receptors such as α6β4, αvβ3, αvβ5, α5β1 and chemokine receptors such as CXCR2 and CXCR4 (Bauer et al., 2007; Mierke et al., 2008c; Teicher and Fricker, 2010; Gong et al., 1997; Ricono et al., 2009; Gilcrease et al., 2009; Sawada et al., 2008). Despite of all these findings, novel approaches such as genomics and proteomics did not fundamentally change clinical outcomes in the field of cancer research. While these biological technologies have not yet fully revealed their potential after the first hype and promotion, they have gained profound insight into fundamental cancer biology, cancer diagnosis and prognosis: the classification and detailed staging of tumors, numerous marker proteins for several cancer-types, and mapping of specific human cancer-types. A main criticism of these novel approaches is that the expression levels of numerous genes and molecules, which are differently regulated during cancer progression depend on the cancer disease stage and how they contribute or regulate cancer progression, is still yet not fully understood. In more detail, these genomic and proteomic based analysis methods ignore the localization of the molecules in special compartments such as lipid rafts (Runz et al., 2008), their activation and assembly state and finally also their life-time, turn over-, modification- and recycling rate (Garcia

*Abbreviations:* MLCK, myosin light chain kinase; RLC, regulatory light chain; EMT, epithelial–mesenchymal transition.

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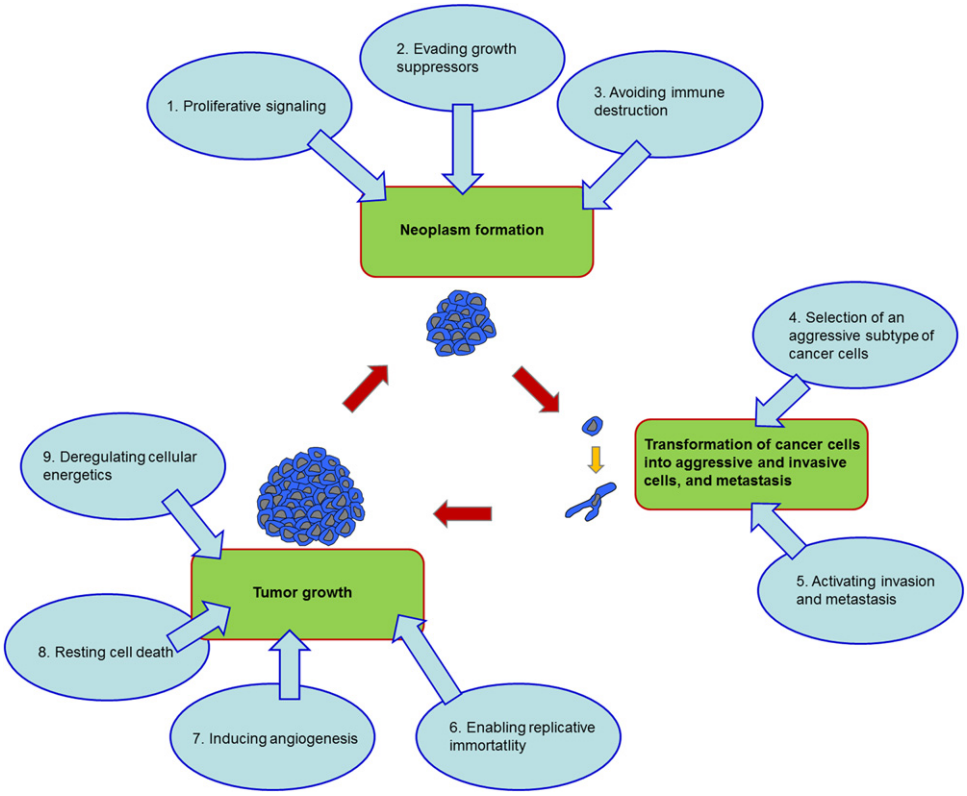


**Fig. 1.** Nine hallmarks of cancer disease. The hallmarks can be summarized into three major groups: neoplasm formation (hallmarks 1–3), transformation of cancer cells into aggressive and invasive cells (hallmarks 4–5) and tumor growth (hallmarks 6–9).

et al., 1998; Gu et al., 2011; Veiga et al., 1997; Liu et al., 2011; Caswell et al., 2008). As current classical approaches have not captured the full complexity of the problem to get more insights into malignant cancer disease progression, adapted classical physical approaches and newly biophysical methods have been developed to be used in the field of cancer research. In particular, these new directions have changed pronouncedly the field of current cancer research and have broken down the classical view on cancer disease. Finally, a ninth hallmark, which includes the aspect of physics into classical cancer research, is that the primary tumor and the tumor microenvironment alter the survival conditions and cellular properties of a certain set of cancer cells, which subsequently favors the selection of an aggressive (highly invasive) subtype of cancer cells. These aggressive subtype of cancer cells may be able to reduce cell–cell-adhesions to neighboring cells, cross the tumor boundary of the primary tumor including the tumor-surrounding basement membrane and migrate into the tumor stroma consisting of an extracellular matrix scaffold and embedded cells. This novel ninth hallmark can be included after the hallmark of avoiding immune destruction and before the hallmark activating invasion and metastasis (Fig. 1). These nine hallmarks can be grouped into three major groups: neoplasm formation (hallmarks 1–3), transformation of cancer cells into aggressive and invasive cells (hallmarks 4–5) and tumor growth (hallmarks 6–9; Fig. 1). This review will focus on the novel ninth hallmark, the selection of an aggressive subtype of cancer cells, which have the capability to down-regulate cell–cell adhesions, possibly up-regulate cell-matrix adhesions and regulate their mechanical properties that facilitate their transmigration through the basement membrane and their migration into the connective tissue and on the hallmark activating invasion and metastasis. These processes will be presented from a physical point of view and thus, break-down the classical view on cancer.

## Focus on the selection of aggressive cancer subtypes as well as invasion and metastasis

In step-by-step series, the progression of the metastatic process evolves, but occurs rarely among the huge number of cancer cells of the primary tumor site. Cancer metastasis is a complex scenario consisting of sequential steps and subsequently is responsible for over 90% of cancer-related deaths. In particular, cancer cells spread from the primary tumor, cross boundaries of the primary tumor, migrate or flow through vastly different microenvironments, including the tumor stroma, the blood vessel endothelium, the vascular system and the tissue at a secondary site (Chambers et al., 2002; Steeg, 2006), where the probability of metastasis is more likely to occur compared to other non-targeted sites (Fig. 2). Together with enhanced cellular invasiveness, cellular morphology, cytoskeletal architecture and biomechanics, cancer cells can remodel and adapt their microenvironment (Brábek et al., 2010; Mierke, 2012; Wolf et al., 2003) to facilitate tumor progression and finally to metastasize in targeted organs.

In addition, it has been shown that the tumor microenvironment is no passive compartment of the cancer disease progression process, it is hence rather an active element, which is critical for providing the physical properties for malignant tumor progression (Engler et al., 2005; Mierke et al., 2011a,b). Thus, the tumor microenvironment seems to be highly critical for all steps of the cancer metastasis process. In particular, the endothelial microenvironment of a tumor is an active element for providing cancer cell invasion into dense 3D extracellular matrices, where the pore-size is smaller than the cancer cell's diameter (Mierke, 2011).

External physical properties of the tumor microenvironment can be of geometric or mechanical nature, as well as differences in the dimensions of the space, which are available for invasive cancer cells (Provenzano et al., 2006; Kumar and Weaver, 2009;