Research

Spheroid Pasta – a Possible Mechanism of Cancer Promotion

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Abstract

In biology, spheroids are three-dimensional clusters of cells that aggregate into a spherical shape. After intercellular interactions, tumor cells generate adhesion molecules like cadherins and integrins on their surfaces, facilitating cell survival and enabling the formation of spheroids as a dense structure. Organoids are three-dimensional, highly complex mini-organs with specific functions. They derive from stem cells or progenitor cells from patient-derived normal or tumor tissue and are used for disease modelling and development. The mechanism of spheroid formation shares profound physical similarities with the globular folding of proteins and interaction between quarks in atomic nuclei. Spherical shape is attained due to minimization of the surface area at constant volume and thereby minimize the energy of interaction within the system. In neutron stars, the interactions between the constituents are predicted to attain peculiar self-assembled shapes that deviate from spherical. These structures are called nuclear pasta. Also in cancer, the shapes of spheroids are often significantly different from spheres seen in healthy cell cultures. In this contribution we introduce the biophysical origin of the so-called Differential Adhesion Hypothesis in spheroids, proteins and atomic nuclei, which states that cells will reorganize in such way to minimize the free energy of the surface and of the internal bonding.

Keywords: Organoid; Cadherins; Breast cancer; Differential Adhesion Hypothesis; Minimal surface at constant volume; Nuclear pasta

1. Spheroids and organoids as models of cancer promotion

In biology, spheroids are three-dimensional clusters of cells that aggregate into a spherical shape (**Figure 1A**). After intercellular interactions, tumor cells generate adhesion molecules like cadherins and integrins on their surfaces, facilitating cell survival and enabling the formation of spheroids as a dense structure (Gunti et la, 2021). Spheroids allow cells to interact with each other and their extracellular matrix in all directions, much like they do inside a living organism. As a spheroid grows, it naturally develops distinct metabolic layers due to limited diffusion of oxygen and nutrients to its center: proliferating zone (outer layer), where the cells divide rapidly, quiescent zone (middle layer), where they stop actively dividing, and necrotic zone (mostly in large spheroids) (Nath & Devi, 2016). Multicellular spheroids, usually composed of established immortalized cell lines, are widely used in cancer research to predict how a patient's tumor will respond to chemotherapy (Lazzari et al., 2017). Organotypic multicellular spheroids (OMSs) are created through very gentle mechanical or enzymatic dissociation of cancer tissues, preserving adjacent non-tumor cells, including stromal, vascular, and immune elements of the tumor microenvironment (TME). Consequently, OMSs, resemble *ex vivo* explants and typically preserve numerous histological characteristics and cellular heterogeneity (D'Antonio & Liguori 2025). Spheroids can be used to study single and collective cell migration and invasion (Blondeel et al., 2025).

Organoids (**Figure 1B**) are three-dimensional, highly complex mini-organs with specific functions. They derive from stem cells or progenitor cells from patient-derived normal or tumor tissue (Rycaj &Tang, 2015) and are used for disease modelling and development (Clevers, 2016). Tumor cells self-assemble and interact with each other to form 3D tissue-like structures, following specific protocols, usually involving Matrigel or other components of the ECM. The presence of ECM components elevates the structural complexity of organoids when compared to spheroids, creating an architecture that is more akin to that of the original tumor (Paolillo et al., 2021).

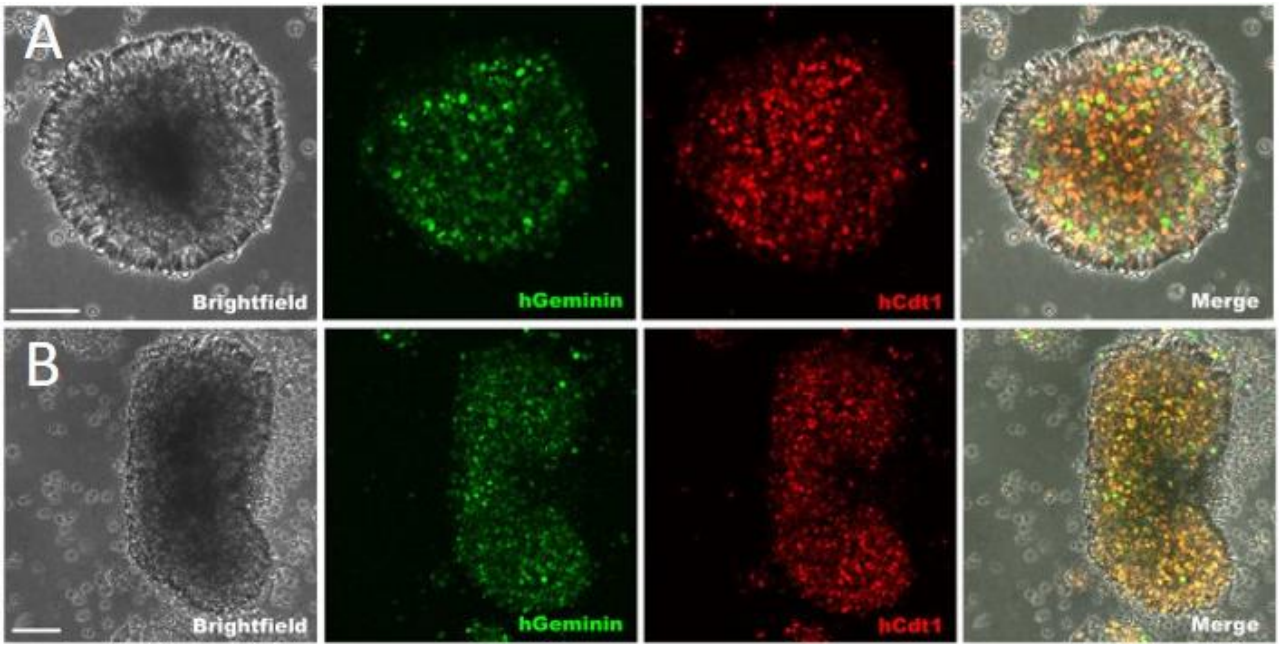

Figure 1. A and B: Confocal microscope images of a spheroid derived from breast cancer line MCF7 cells. Spheroids (A) and rods (B) with cells are geminin-positive (green) or CDT1-positive (red). Scale bars represent 100 microns. From (Haspels & Kuijten, 2024, under CCBY 4.0 licence).

Scientists use CRISPR-Cas9 genome editing to introduce defined initiating mutations, such as APC and KRAS, into human intestinal stem cell–derived organoids to model early tumorigenesis (Drost et al., 2015). They then monitor how these cells respond to growth factors or inflammatory signals that act as promoters, driving the cells toward invasive cancer (Hanahan & Weinberg, 2011). Also they study the roles of E-cadherin and cytoskeleton on formation of spheroids (Smyrek et al., 2019). In the promotion phase, cells that have undergone initiation require external support to become fully cancerous (Huang, 2015).

Three-dimensional models allow researchers to add specific components of the tumour microenvironment, such as cancer-associated fibroblasts or inflammatory cytokines, to see how they promote the malignant transformation of these initiated cells (Nath &Devi, 2016; Franchi-Mendes et al., 2021).

2. Mechanism of formation of spheroids

Hypothetically, spheroids can be formed by self-assembly where cells transition from a disorganized suspension into a highly structures three dimensional lump (Cui et al., 2019). This mechanism is primarily driven by the Differential Adhesion Hypothesis which states that cells will reorganize in such way to minimize the free energy of the surface and of the internal bonding (Athanasiou et al., 2013). Biologically the process starts with loose aggregation of dispersed cells which are drawn together by interactions between membrane integrins and long chain extracellular matrix fibers to create a weak unstable cluster (Lin et al., 2006). Then the cells up-regulate and accumulate cadherins at the cell surface to enable direct cell-cell bonding (Maître & Heissenberg, 2013). Cadherin-cadherin interactions pull cells closer together which is followed by reorganization of the actin cytoskeleton (Nelson et al., 2008). Actomyosin contractility provides the internal tension necessary for the cluster to round into a sphere. Spheroid formation is a self-assembly mechanism driven by the transition from integrin-mediated cell matrix adhesion to cadherin-mediated cell-cell compaction (Lin et al., 2008). Furthermore, the shape of the spheroid, cell sorting, tissue spreading and segregation are guided by minimization of the free energy as cells tend to maximize their mutual binding (Foty & Steinberg, 2005).

3. Coalescence – a general mechanism of compaction in organoids, proteins and atomic nuclei

The mechanism of spheroid formation shares profound physical similarities with the globular folding of proteins. While spheroids involve whole cells and proteins involve single chains of amino acids, both processes are driven by the same physical principle – minimization of the free energy. While the primary driver in organoids is differential adhesion, protein globular folding is subjected to hydrophobic collapse (Brylinski et al., 2006); while the surface force in organoids is cortical tension by actomyosin (Scheibl & Heinz, 1999), in protein folding, the parallel effect can be provided by water exclusion; while the attractive interaction between the cells in organoids is exerted by cadherins (Arslan et al., 2021), in proteins, the attractive interactions between the chain building blocks are Van der Waals interactions, electrostatic interactions and hydrogen bonds (Xie et al., 2015); while the intermediate state of the organoids are loose aggregates, in proteins, there are molten globules (Bychkova et al., 2022). In both systems, unstable parts are hidden from the surrounding fluid. In spheroids, cells with strongest adhesion (rich with cadherins) migrate to the center to maximize mutual contact, while less adhesive cells remain on the surface (Foty & Steinberg, 2005). In proteins, hydrophobic amino acids collapse toward the core to hide from water, and hydrophilic parts stay at the surface (Sun, 2022). Both processes are spontaneous self-assembly events. Spheroids do not require a scaffold. They form naturally when cells are kept in suspension and are allowed to collide via Brownian motion or gravity. Proteins fold into globular state based on their amino acid sequence. Both systems minimize the energy and thereby transform a disorganized group of parts into a compact three-dimensional globular shape.

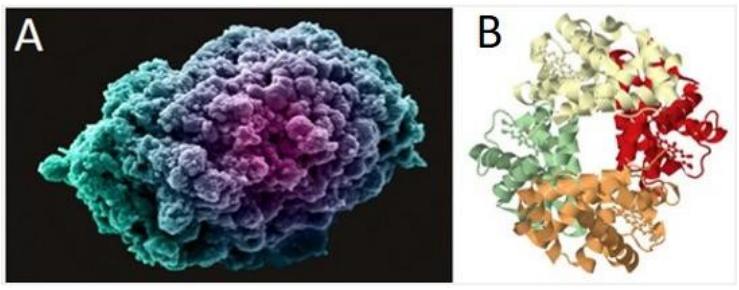

Figure 2. A: Cancer cell spheroid imaged by confocal microscope (Al Jamal et al., n.d., CCBY 4.0 licence), B: reconstructed shape of deoxy haemoglobin derived from Protein Data Bank entry 11LFL (Lenov, 2013, CCBY 3.0 License).

The physical mechanism of spheroid formation can be applied to atomic nuclei contained in the Liquid drop model (Hein et al., 2022). While cellular spheroids are held together by cadherins, atomic nuclei are held together by the strong nuclear force (Lin et al., 2006). Despite the difference in scale, the physical mechanism that explains the globular shape is the same. Just like a spheroid, the atomic nucleus minimizes its surface area to reach its minimal energy state. In organoids, cells on the surface have "missing" neighbours, which creates surface tension pulling towards the center until the structure is compact. In atomic nuclei, protons and neutrons on the surface have fewer neighbours to bond with via the strong force which is energetically unfavourable. To minimize the surface, the nucleus becomes compact. In organoids, the repulsive force between the cells derives from mechanical crowding, in the atomic nucleus, the parallel force is the Coulomb repulsion. In both systems, the shape is a delicate balance between aggregation and repulsion. In a spheroid, if the cells grow too large, nutrient deprivation at the core causes necrosis. In the atomic nucleus, as it gets larger, the Coulomb repulsion between protons starts to overcome the strong force. Spheroids can dissolve into individual cells if the cadherins are removed (Lin et al., 2006; Nelson et al., 2008). At extremely high temperatures or densities the nucleons dissolve into quark-gluon plasma (Shuryak, 1980).

The mechanism of coalescence is fundamentally the same in all three above systems of different scales. The liquid drop model of the atomic nucleus treats nucleons as a dense incompressible fluid where surface tension drives the formation of the globular shape, to mirror the self assembly of spheroids and folding of proteins.

4. Sphere as the shape of minimal surface at fixed volume

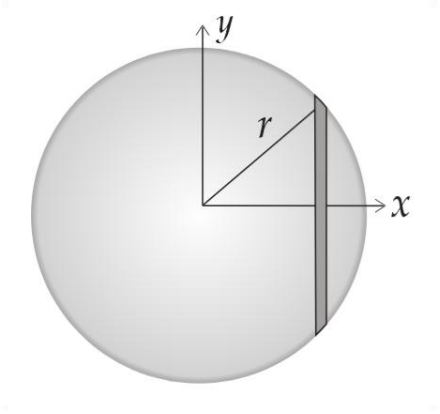

Figure 3. Parametrization of the shape. The three dimensional shape is obtained by rotation of $y(x)$ around the x-axis. The shaded area represents the slicing of the shape in constructing the area and volume elements.

We consider the axisymmetric body obtained by rotating a function $y(x)$ around the x-axis (**Figure 3**). The area element for such shape is

$$\mathrm{d}S = 2\pi y(1+y'^2)^{1/2}\mathrm{d}x\,, \qquad (1)$$

and the volume element is

$$\mathrm{d}V = \pi y^2 \mathrm{d}x \quad . \qquad (2)$$

We are looking for the extreme area at given volume, for which we construct a functional

$$G = \int 2\pi y(1+y'^2)^{1/2}\mathrm{d}x + \lambda \int \pi y^2\,\mathrm{d}x = \int L\,\mathrm{d}x \qquad (3)$$

where

$$L = 2\pi y(1+y'^2)^{1/2} + \lambda\,\pi y^2\,. \qquad (4)$$

and λ is the Lagrange multiplier. The solution of the variational problem is given by the solution of the Euler-Lagrange equation

$$L_y - \mathrm{d}L_{y'}/\mathrm{d}x = 0 \quad . \tag{5}$$

Performing the derivation yields

$$1/(1+y'^2)^{1/2} - y'y''/(1+y'^2)^{3/2} + \lambda\, y = 0 \quad . \tag{6}$$

The ansatz

$$y = (r^2 - x^2)^{1/2} \tag{7}$$

yields

$$y' = -\, x/y \quad , \tag{8}$$

and

$$y'' = -\, r^2/y^3 \quad . \tag{9}$$

Inserting (7) – (9) into (6) gives

$$2y/r - \lambda\, y = 0 \quad , \tag{10}$$

so that the ansatz (7) solves Eq.(6) when

$$\lambda = 2/r \quad . \tag{11}$$

The ansatz describes a circle with the centre at the origin of the coordinate system and radius r meaning that the rotational body is a sphere with the centre at the origin of the coordinate system. Eq.(6) can be solved also by the ansatz y = const representing an open cylinder, however, this solution corresponds to a local minimum of the surface area.

5. Compactoids: spheroids, tuboids and toroids

While the word "spheroid" specifically implies a sphere, the compactoids can attain also other shapes. Just as a spheroid can be squashed into an ellipsoid (Wallingford et al., 2013), an atomic nucleus can become prolate or oblate due to its internal spin of nucleons (Matejska-Minda et al., 2019). In biology, some globular structures naturally elongate into tubes during development. For example, the neural tube or vascular sprouts being as clusters but use convergent extension (cells crawling over each other to lengthen the tissue) to move from sphere-like to a tube-like state. While spherical shapes are the default for minimizing the surface area at fixed volume, tubular structures are achieved when the individual building blocks have a specific geometric curvature or directional bonding that favors tubular or toroidal shape, for example cytoskeletal microtubules (tubular)(Gerhardt et al., 2003; Keller et al., 2000), green fluorescent protein (tubular)(Yang et al., 1996) and membrane channels (toroidal). In the preparation of such assemblies, biological systems make extensive use of self-assembling and self-organizing strategies (Bong et al., 2001). Stable tubular self-assemblies exist and are essential for cellular life. Their stability is maintained by building blocks designed with directional instructions that favour cylindrical or toroidal curvature over the spherical one.

Tubular atomic nuclei were predicted to be under the extreme gravitational pressure found in the inner crust of neutron stars (Caplan & Horowitz, 2017). In this environment, atomic nuclei are compressed so tightly that they lose their spherical shape and self-assemble into exotic, nonspherical configurations collectively known as "nuclear pasta". As density increases toward the center of a neutron star, the competition between nuclear attraction and Coulomb repulsion yields complex, non-spherical patterns such as tubes,

sheets and bubbles (**Figure 2**); these configurations minimize their energy (Caplan & Horowitz, 2016). The name "nuclear pasta" arose due to a resemblance to different varieties of pasta — such as lasagna, gnocchi and spaghetti.

Historically, a sequence of nuclear pasta shapes was predicted to appear in the deepest region of the inner crust of a neutron star within the compressible liquid drop picture, when the filling fraction exceeded some threshold values, however, later calculations showed that stability of different shapes could be explained by the nuclear curvature correction (Shchechilin et al., 2025). To mathematically describe the pasta shapes, Minkowski functionals were used (Lopez et al., 2025). It was suggested that volume V, surface area S, mean curvature H and Euler characteristic χ (a topological number that counts handles and holes) uniquely quantify the shape. The "decision" of which shape to form is governed by the minimization of the surface and Coulomb energy density

$$\varepsilon = \varepsilon_{surface} + \varepsilon_{Coulomb} . \quad (12)$$

Minkowski functionals provide the mathematical description of a three dimensional shape allowing scientists to track how spheroids, organoids and nuclear pasta evolve from globular to tubular form.

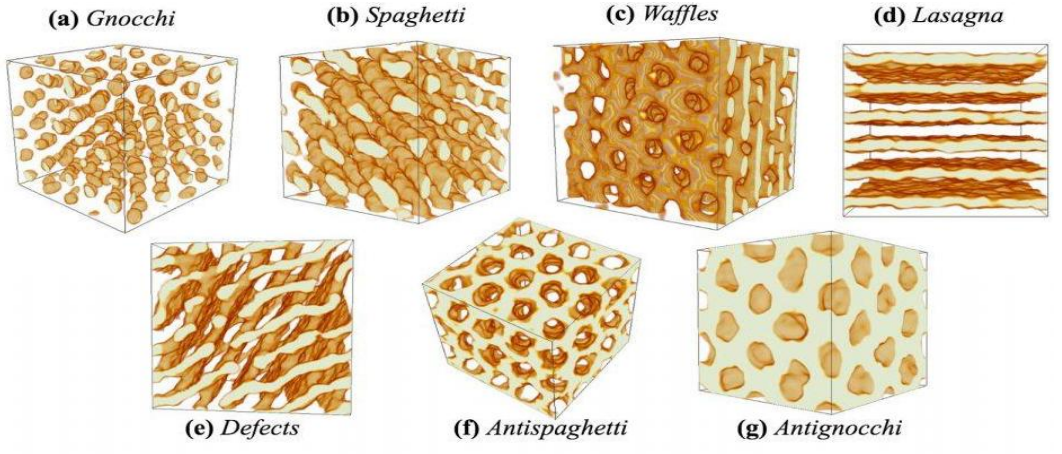

Figure 2. Examples of different nuclear pasta phases. From (Caplan & Horowitz, 2016, with permission).

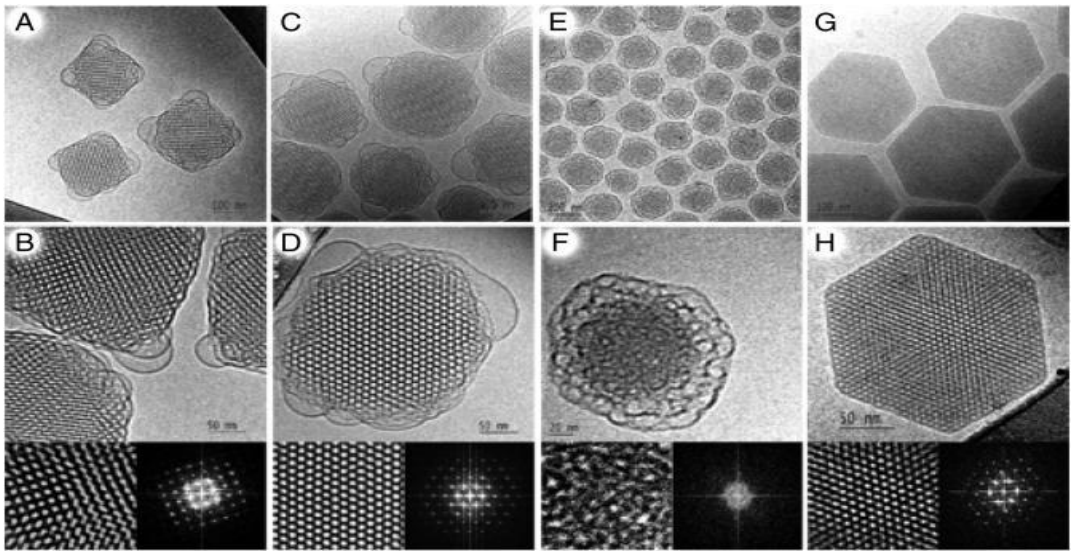

Figure 3. A-H: Micrographs of nonlamellar lipid phases. From (Barauskas et al., 2005, with permission).

6. Shape of compactoids as indication of the clinical status

In cancer, the shapes of spheroids are often significantly different from spheres seen in healthy cell cultures. While healthy cells tend to form highly symmetrical compact balls, cancer spheroids can become irregular, fuzzy at the edges and elongated depending on the malignancy and the environment (Trivedi et al., 2021). While healthy cells are rich with cadherin and tightly packed, cancer cells are loose, exhibit blebbing and protrude invadopodia into the matrix (Sibony-Benyamini & Gil-Henn, 2012). Researchers use these shape differences as a diagnostic tool to measure how aggressive a cancer is.

Aggressive cancer cells undergo epithelial-to-mesenchymal transition (Ribatti et al., 2020). They lose E-cadherin and start crawling from the center (Kalluri & Weinberg, 2009). This causes the smooth globular shape to develop spikes or tendrils as the cancer begins to invade the surrounding extracellular matrix (Tarin & Croft, 1969; Shah et al., 2024). In some cancers, the spheroid becomes ellipsoid (oval)(Cheng et al., 2009). This can be caused by mechanical stress. Cancer cells generate higher internal pressure pushing the spheroid into an elongated shape (Cheng et al., 2009). If the surrounding tissue is stiff (like in breast cancer) the spheroid may flatten or stretch along the stiffest fibers (Jahin et al., 2023). Highly aggressive cancer spheroids can become unstable. Instead of one large ball, they might break into several smaller satellite clusters. This is a mathematical manifestation of low surface tension. The attractive interaction is not strong enough to hold the globular shape. Minkowski functionals show a drop of cancer cell spheroid sphericity ϕ,

$$\phi = \pi^{1/3}(6V)^{2/3}/S, \tag{13}$$

where $\phi = 1$ for the sphere (Cruz Matias et al., 2018), to 0.7 or lower as the cells become more invasive. Euler characteristic χ increases with appearance of holes which indicates a more advanced state of the disease. In cancer, globular geometry is often lost. The transition from a smooth sphere to a spiked or irregular shape is a direct visual indicator of cancer promotion and metastatic potential.

7. Discussion

In the context of cancer biology, E-cadherin is a tumor suppressor protein that acts as a glue between cells. When cancer cells lose E-cadherin, they become loose, nonspherical and capable of invading other tissues – a process called epithelial–to-mesenchymal transition. Physiotherapy helps normalize the environment around a tumor which supports the healthy function of E-cadherin. It reduces hypoxia which is a major trigger for the loss of E-cadherin. By improving the blood flow and oxygen delivery, physiotherapy reduces the hypoxic drive that normally would shut down E-cadherin production. High lactate levels make the tumor environment acidic, which impairs cell adhesion. Exercise helps clear lactate and normalize pH, creating a state where E-cadherin can function more effectively. Physiotherapy and exercise have been shown to increase E-cadherin levels and stabilize its activity, which helps clear lactate, lock cancer cells in place and prevents them from transforming into a more invasive migratory state.

E-cadherin is a brake on vesiculation of cells. High levels of E-cadherin are associated with lower rates of extracellular vesicles shedding, in particular large vesicles that bud directly from plasma membrane. By maintaining stable adherens junctions, E-cadherin prevents the cellular stress states that typically trigger the release of stress-induced extracellular vesicles.

In breast cancer pathology, E-cadherin is much more than a protein. It is a primary diagnostic marker used to distinguish between the two most common types of breast cancer: ductal and lobular. Ductal carcinoma are usually E-cadherin positive. The cells maintain strong glue between them, forming clusters, tubes, or solid sheets of cells. Lobular carcinoma are defined by loss of E-cadherin. Without this glue, the cells become discohesive and migrate through the tissue in a characteristic single-file pattern (Pai et al., 2013). In lobular breast cancer, the loss of E-cadherin is typically an early, defining event caused by mutations in the CDH1 gene (Bai & Troxell, 2025). It was reported (Shah et al., 2024) that in mixed ductal and lobular carcinoma, the ductal and lobular regions revealed differences in transcriptomic, genomic, and single-cell properties. Genetic and epigenetic inactivation of CDH1 was found in lobular but not ductal regions (Shah et al., 2024). In some cases, the

gene is physically present but silenced by chemical changes (methylation) that prevent the cells from reading the DNA instructions. While E-cadherin is a tumor suppressor that prevents cells from breaking away, its role in later stages is surprisingly complex. In ductal cancers, losing E-cadherin is a sign of epithelial to mesenchymal transition, making the tumor more aggressive and likely to spread. However, metastatases re-express E-cadherin (Rubtsova et al, 2022).

5. Conclusions

Across different scales, we can find similar features subjected to the same natural laws. Geometrical properties and interaction between building blocks seem to be found at the origin of different phenomena, including cancer promotion that is the scope of this paper. However, due to similarities in fundamental mechanisms, the results obtained in studying other systems such as protein folding and gluon-quark plasma in neutron stars may be useful in designing the diagnostic methods for specific diseases. Theoretical physics is an essential tool for describing organisms because it provides the mathematical and conceptual framework needed to understand how complexity emerges from simpler parts. While biology focuses on the what and where of life, biophysics and physics of complex systems focus on the how – speciffically how physical laws govern the movement, energy, and information that make life possible.

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