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Review Article

TRANSFORMED CELL - THE UNUSUAL SUCCESSION OF A USUAL CELL

Sanjeet Kumar Das*[1,2], Arpita Maitra[1], Mousumi Pal[2], R.R. Paul[3]
[1]*Ph.D. Scholar, Department of Oral and Dental Sciences, JIS University, Kolkata, India*
[2]*Department of Oral and Maxillofacial Pathology, Guru Nanak Institute of Dental Sciences and Research, Kolkata, India*
[3]*Department of Oral and Dental Sciences, JIS University, Kolkata, India*
**Corresponding author: dassanjeetkr@gmail.com*
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ABSTRACT
After the first observations of life under the microscope, it took almost two centuries of research before the idea that all living things are composed of cells or their products was speculated. The development of the microscope was a requirement for the discovery of cells. In 1673, the Dutch botanist, Anton van Leeuwenhoek, reported seeing a myriad of microscopic "animalcules" in water. Over several decades, knowledge about the structure and functions of the cell has progressed tremendously due to the advancement in various techniques like next generation sequencing, and genome wide analysis. Cancers are generated from normal cells by random karyotypic rearrangements. Immortality is a common characteristic of cancers, but its origin and purpose are still unclear. Since such rearrangements disturb long-established mitosis genes, cancer karyotypes vary instinctively but are stabilized perpetually by clonal selections for autonomy. The differentiation stage of tumors is a vital aspect in the histopathological classification of solid malignancies, strongly associated with tumor behavior, as an immature tumor is more aggressive than the more differentiated counterpart. The central focus in these events is the cell that undergoes a series of morphological and biochemical changes in course of its transition from normal to a transformed tumor cell; thus acquiring typical characteristics which aids in the process of progression. In this review an attempt has been made to enlighten upon the unusual behavior of a normal cell in transition.

Keywords: Transformation, Malignant, Tumor cell, Nuclear cytoplasmic ratio, Genomics.

1. INTRODUCTION
Life is a dynamic process, its basic structural and functional unit being the Cell. The conceptual development of life and the cell has witnessed several milestones in recent history. *Micrographia* by Robert Hooke published in 1665, being the first major publication of its kind, from the Royal Society, inspired and established the importance of microscopy. Later, Theodor Schwann's *Cell Theory* in 1839 which was supported by Sir Virchow, stated: all organisms consist of one or more cells; Cell is the basic unit of structure for all organisms; all cells arise from preexisting cells: thus was the significance of cell biology established [1, 2].
Concepts from comparative genomics that analyses nucleotide sequences of genomes, divide life form into two major divisions-the cellular and the viral empires. Carl Woese in a revolutionary study in 1977 compared the small subunit of ribosomal RNA molecule in cellular life form in light of their nucleotide sequencing and then the three domains of life emerged namely-Bacteria, Archaea and Eukarya. The Eukaryotes are regarded as "archaebacterial chimeras" that is hypothesized to have evolved following an endosymbiotic event that gave rise to specific cellular organelle. The Prokaryotic host cell incorporates another prokaryotic cell; each one having its own genome. The incorporated cell replicates within the host cell. A resultant replication error or lysis of the cell leads to incorporation of its genome with that of the host cell; which becomes a blend of both genomes, being enclosed in an endomembrane that eventually evolves into the nucleus, thus typifying the Eukaryotic Cell [3, 4].
Besides nucleus, the chloroplast and mitochondria are important facets of eukaryotic cell; the later is supposed

to have developed from engulfed aerobic bacterium that has formed an endosymbiotic relationship with the host; which by engulfing photosynthetic bacteria led to evolution of the chloroplast organelles [5].

Thus a eukaryotic cell has evolved into a 10-100 micron diameter entity with the presence of cytoskeleton, cytoplasmic organelles and multiple linear DNA molecules consisting of about $1.5X10^7$ to $5X10^9$ base pairs. Biochemically the cell is a constellation of various macromolecules [6].

2. BIOCHEMICAL COMPOSITION OF CELL- AN EVOLUTIONARY PERSPECTIVE

With the first life on earth, the atmosphere is hypothesized to contain little or no free oxygen, but rich in carbondioxide and nitrogen and gases like hydrogen, hydrogen sulphide and carbon monoxide. Such reducing environment had triggered the formation of the first organic molecules, given a source of energy such as sunlight or electrical discharge. Stanley Miller (1950) experimentally documented the formation of organic molecules even amino acids [7].

The plausible prebiotic conditions had given rise to monomeric building blocks of macromolecules that have been demonstrated to spontaneously polymerize. The critical character of the macromolecule had been the ability to replicate, thus signifying the nucleic acids as the first informational macromolecule directing its own self replication; along with the proteins [7, 8].

The primary composition of cells is water, inorganic ions and carbon containing organic molecules. Water is the most abundant accounting for about 70 percent of total cell mass. Hence, the biochemical milieu is the result of interaction of water molecules with the other water cellular constituents. Its criticality is defined by its polarity, when hydrogen atoms have slight positive charge and oxygen has a slight negative charge [6, 7].

Inorganic ions primarily consist of sodium, potassium, magnesium, calcium, phosphate, chloride, bicarbonate-constituting less than 1 percent of cell mass. The organic constituents belong to one of the four classes-carbohydrates, lipids, proteins and nuclei acids. These are basically macromolecules found by polymerization on low molecular weight precursors-amino acids, nucleotides, simple sugars etc.; constituting about 80-90 percent of dry weight of cells [6].

Carbohydrates include simple sugars, intake of glucose as well as polysaccharides. Their breakdown provides a source of cellular energy and precursor for other cell constituents. Polysaccharides form the structural components of cells. They also serve as marker for multiple cellular recognition processes like cell to cell adhesion and protein transport to intracellular destinations. Glycosidic bonds between the carbon atoms are defined by dehydration reaction with mono-saccharides forming the polymers [6].

Glycogen is the primary storage form of carbohydrate in animal cell. In addition to energy storage these polymers are often linked to proteins playing important roles in protein folding and serve as molecules on cell surface, thus playing important role in cell recognition and interaction between cells [6, 8].

Lipids, in their simplest forms are fatty acids, consisting of long hydrocarbon chain (16/18 carbon atoms) with carboxyl group at one end. The long hydrocarbon chains contain the C-H bonds that reveal their hydrophobicity, being unable to interact with water; and bearing significance in maintaining the integrity of biological membranes. In addition to energy storage, they are active in cell signaling both as steroid hormones and as messenger molecules, converging signals from cell surface receptors to intracellular targets. The storage forms of lipids are triacylglycerols or fats which when required can be broken down for use in energy yielding reactions. Cell membranes are chiefly composed of phospholipids along with glycolipids and cholesterol, which are biochemically amphipathic molecules. The steroid hormones are derivatives of cholesterol, which are diverse group of chemical messengers containing four hydrocarbon rings with distinct functional groups attached [6, 9].

The nuclei acids in the form of DNA and RNA act as principal informational molecules functioning as the genetic material. They are chiefly nucleotide polymers consisting of purine and pyrimidine bases linked to phosphorylated sugars. The messenger (mRNA) serves as a template for protein synthesis; thus protein execute the task, the information of which is carried by the nucleic acids [6, 10].

Proteins are a diverse group of macromolecules functioning as the structural components, playing part in storage and transport of small molecules while providing defense. The constituent polymers are formed by different amino acids that consists of a carbon atom bounded to a carboxyl group, an amino group, a hydrogen atom and a distinct side chain; and are joined together by peptide bonds between alpha amino group of one group and alpha carboxyl group of the second. Thus polypeptides are formed consisting of hundreds to few thousands of amino acids; containing two distinct ends,

one terminating into alpha amino group/N terminus while the other in alpha carboxyl group/C terminus. Specific proteins are polypeptides with specific amino acid sequences. Proteins generally exhibit four levels of structural organization. The primary structure is the sequence of amino acids in its polypeptide chain. The secondary structure is the regular arrangement of amino acids within localized regions of polypeptides into alpha helix and beta sheets; which are held together by hydrogen bonds between CO and NH groups. Tertiary structure results from interactions between side chains of amino acids lying in different region of the primary sequence, creating compact globular domains. The fourth organizational level, the Quarternary structure results from interactions between polypeptide chains. Such organizational stratifications bears testimony to the diversified and complex macromolecular structure of proteins [6, 11].

3. ALTERATION OF CELLULAR PLASTICITY AS A PREAMBLE TO MALIGNANT TRANSFORMATION

The molecular and phenotypic changes acquired by cells during malignant progression and transformation are collectively known as Cellular Plasticity. Alteration or loss of normal cell identity and function accompanies cancerous change termed as initiation. The tumor cells under various intrinsic and extrinsic influences ultimately form a "volatile microenvironment" which is orchestrated by genetic, epigenetic or transcriptional fluctuations. At cellular level the changes usually manifest as "transdifferentiation" while at the tissue level it is called "metaplasia" for essentially endoderm derived tissues that is associated with predisposition to development of malignancy. Selective proliferation, stem cell lineage differentiation and drop out of certain cell types often predispose to additional metaplastic changes [12, 13].

It is associated with large alteration in chromatin landscapes, leading to changes in gene expression. Differentiation alterations have been proposed to be more fine-tuned by the process of Epithelial to Mesenchymal Transition (EMT), wherein epithelial cells lose their intercellular connections chiefly fibroblasts. Evidences of 'complete' EMT is now supported by "partial EMT" status which encompasses dual expression of mesenchymal and epithelial genes and their interplay at both transcriptional and protein levels [14, 15].

Such events lead to various morphological and biophysical alterations in the transformation and induction of metastasizing property to the transformed cells.

The chief criteria to define nature of transformed cell are a) immortality b) ability to form malignant tumors on transplantation into host system. Neoplastic state was defined as the ability of cells to grow progressively into invasive, serially transplantable neoplasms [16, 17].

The progressive changes pertaining to malignant change of cell include increased cytoplasmic basophilia, increased number and size of nucleoli, increased nuclear cytoplasmic ratio, retraction of cytoplasm, formation of clusters and cords of cells [16, 18].

The properties of cells with malignant phenotypes are less differentiation, rapid growth and progressive atypia [18].

Lack of differentiation gives rise to the concepts of anaplasia and dedifferentiation. The cancer cell usually regresses to a lesser degree of differentiation. The tumor cell is believed to arise in a state of low differentiation. The cumulative effect of such differentiation leads to the episode of tumor progression [19].

Increased tendency for growth reflects at the cellular level by increased cytoplasmic basophilia and increase in size and number of nucleoli; with an abnormal nucleolar organizer region [18, 20]. Higher glycogen content resembles the physiology of embryonic cells; when expressed in mature cells correlates with anaerobic glycolysis, typical of both embryonic and tumor cells [21]. The proteins at the nuclear pores called Nucleoporins, forms an intense cellular traffic; antibodies to which have been found in malignant tumors and dysplasias [22].

Degree of aggressiveness is also correlated to the features of cellular atypia. Transformed cells appear to be more rounded and irregular correlated with the cytoskeletal disturbances, related to the oncogenes that tend to localize along cell membrane and alter the organization of the attachment plaques [23].

Moreover, the nuclei of the cell also vary in size and shape owing to the abnormal chromosomes, ring-shaped, dicentric, etc. and bear significance to genetic instability. Similarly, abnormalities in centrosomes that is losing association with patent tumor suppressor gene p53, provokes centrosomal replication beyond physiological turnover cycles. Multiple centrosomes may lead to multipolar mitosis often leading to errors, giving a mutant phenotype [24].

4. ALTERED PROPERTIES OF TRANSFORMED CELLS

4.1. Immortality

According to the immortalization theory, "Cells that have stabilized their telomeres through the actions of telomerase or the ALT mechanism proliferate indefinitely and are therefore said to be immortalized. Cell immortalization is a step that appears to govern the development of all human cancers." [26] Physiologicaly, linear chromosomes ought to be capped by telomeres and progressively shortened when reconstituted by telomerase. Tumor cells express telomerase, which in association with immunostabilizing oncoprotein Myc may lead to the evasion of senescence [25].

4.2. Loss of anchorage dependence

Orchestrated by cytoskeletal changes transformed cells have shown growth in fluid media without attachment to substrate [26, 27].

4.3. Loss of contact inhibition of proliferation (CIP)

Contact inhibition resembles non-cancerous cells to cease proliferation and growth on coming in contact to each other. High cell density or soft extracellular matrix promote such inhibition. But on malignant transformation, this property is lost leading to uncontrolled proliferation, neoplasm formation and metastatic potential. CIP is reversed in physiological condition requiring rapid cell growth, such as embryonic development, wound healing or tissue regeneration [28].

The signals of contact inhibition or shape deformation generated by the extracellular matrix, has been linked to "HIPPO" signaling- consisting of kinases and transcriptional regulators [28, 29].

4.4. Loss of orientiation

Organized structures for epithelial cells are a result of robust cell wall, cell matrix interaction with permeability barrier. The asymmetric division of protein, lipid and RNA molecules leading to property of polarity, thus organizing the cell membrane into discrete compartments. Pax and Scribble polarity complex function synchronously maintaining apical basal polarity. Epithelial cancers are marked by the early event of loss of apical basal polarity from pre invasive stages [30].

4.5. Growth factor requirements

The immediate surroundings of a cell provide fate determining signals to the cell under normal conditions. Tissue homeostasis is the result of integration of these signals. The various growth factors of mention are TGF alfa, PDGF, EGF, etc. These growth factors are compact polypeptides binding to transmembrane kinase activity and stimulate specific combination of intracellular signaling pathways as MAPK, PI3K, PHOSPHOLIPASE C GAMA, STATS/SMADS [31].

In physiological scenario, growth factors act by paracrine stimulation, while in oncogenesis, growth factors play a role in fixation of oncogenic mutations, leading to clonal expansion [32].

Abberant growth factor signaling facilitates putative precursor lesions to transfer to cancers by accelerated intraepithelial proliferations [33].

Growth factors play important role in basement membrane disruption, tissue invasion, intravasation, extravasation and metastatic colonization. These factors induce angiogenesis by VEGF, FGF, TGF beta and thereby regulate vasculogenesis and angiogenesis [31].

4.6. Alteration in behavior and function

4.6.1. Chemotaxis and Motility

Chemotaxis can be defined as the phenomenon in which the movement of cells is directed in response to an extracellular chemical gradient. Though an integral part of different physiological process, its role in cancer is chiefly favouring dissemination of cancer cells. The synchronized and stochastic process of invasion, intravasation, extravasation, seeding and growth pave the path favoring dissemination. Chemotaxis of neoplastic cells is chiefly mediated by chemokines, growth factors and Growth Factor Receptors. Tumor associated inflammatory and stromal cells also undergo specific chemotactic migratory movements to facilitate dissemination of cancer [34, 35].

Directed migration of cells has been observed in form of chemotaxis, haptotaxis, electrotaxis and durotaxis. Chemotaxis is accomplished by three steps- chemosensing, polarization and locomotion. Directed migration can occur in groups or single cells. Single cell migration can be reclassified as amoeboid migration or mesenchymal migration. Various methods used in studying migration are-in vitro cultured cells, 3D cell cultures using ECM gels, multiphoton microscopy of live cells. Multiphoton microscopy is used for amoeboid movement study of cancer cells [34-36].

4.6.2. Surface related changes

4.6.2.1. Decreased intercellular Adhesions

DR Coman of Philadelphia (1944) showed that carcinoma cells easily pull apart than their normal

counterpart. Tissue homeostasis relies on cell to cell adhesion and cell to matrix interactions; the modulating proteins being known as "CELL ADHESION MOLECULES"; which are divided into Cadherin, Integrin, Selectin and IgGs [37]. During malignant transformation, cells lose their dependence on integrin mediated interaction and signallings. Promoter methylation, transcriptional repressors, or direct genetic mutation of E Cadherins, result in their loss of expression with the cancer cells gaining a migratory phenotype [38]. Moreover, disassembly of hemidesmosomes in cancer cells, due to lack of polarity by actin protrusions also aid in cell migration and invasion [39]. Down regulation of E- Cad, alfa6beta4 integrins and ECAMs is orchestrated by factors Twist related protein 1, Zinc finger E box binding homeobox protein, transcriptional repressors SNAIL, and SLUG that promote expression of N Cadherin, bta1 and beta2 integrins, akin to malignant phenotype [40].

4.6.3. Altered intercellular communication

In the words of Mc Crea, cell cell communications is "the music that the nucleus hears" which when goes abnormal, then aberrant communication may damage the health of the organism. Communication means sharing of information by different signaling mechanisms. Direct communication within cells Intracrine/Autocrine or among vicinal cells is Juxtacrine. Indirect communication or short distance is Paracrine/Synaptic or long distance is Endocrine. Complex and heterogeneous diseases like cancer encompasses different feedback loops, both initiating or inhibiting different communication pathways. Acute or chronic pathological stimuli interact with the surface proteoglycan layer- glycocalyx of the cell, that consists of five classes of adhesion molecules- immunoglobulins, integrins, selectins, CAMs, cadherins. Within the ECM, endothelial cells and vascular smooth muscles can communicate with each other through CONNEXINS; which work in connection with vascular structures. Thus the communication between glycocalyx, underlying cell structures and ECM is important which is altered in cancers. Reduced level of connexin expression has been correlated with cancer grade progression thus the coupling is lost along with vascular homeostasis in the underlying ECM [41].

4.6.4. Increased susceptibility to agglutination

Cancer research has shown that carbohydrate binding proteins recognize and agglutinate malignant cells more easily than non malignant cells. This gives a clue that tumor cells have specific carbohydrate sites which are more easily expressed and hence quickly recognized [42, 43].

4.6.5. Molecular Shedding from cell surface

Proteins, glycoproteins, enzymes, etc. are shown to shed from the tumor cell surface and has many clinicopathological implications; such shedding is known as Ectodomain Shedding [44]. Measurement of these soluble receptors, catalytic sheddases, proteases, etc. usually correlate with disease states or can be used for monitoring the disease or treatment outcome [44]. Circulating levels of HER2 ectodomain in HER2A breast cancer, plasma levels of RTK sheddase substrates like Mer TK, AXL, MET are detected and monitored in BRAF mutant melanomas; etc. have been hinted. Activation of the shedding of receptors by mechanical cues, hypoxia, radiation, and phosphor signaling offers insight into the mechanism of drug resistance and prognosis assessment. Receptor proteolysis can be detected in fluids of patients with melanoma, glioblastoma, lung cancer, etc. Shedding collagenases can help malignant cells to work their way through the stroma. Shedding fibronectin lead to exaggerated clotting. Shedding plasminogen activator generates plasmin that can help extract cells from fibrin clot [43, 45].

EGFR activity chiefly depends upon the proteolytic shedding of its ligands-TGFbeta, Heparin binding EGF. Shedding leads to diffusion of soluble ligands and resultant stimulation of neighbouring cells in autocrine/ paracrine manner [45].

5. CONCLUSION

The cancer cell in its path of initiation to progression, goes through a long tenacious journey, leading to various molecular and biochemical alterations; that helps the transforming cells to pave their path amidst both conducive and non conducive environments. Both genotypic and phenotypic parameters play important roles in progression of cancer. Changes manifest both at surface levels and also intrinsic pathways. So transformation is a complex process harnessed by a plethora of molecular mechanisms and is therefore a subject of advanced research in future era.

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Conflicts of interest

Nil

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