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ABSTRACT

Understanding the biology of cancer is one major advanced achievement in approach for cancer remission. Although discovery of 10 hallmarks of cancer has played a beneficial role towards this, guiding the understanding of cancer development, metastasis and drive to rational drug design, hallmarks of cancer may not have ended. Besides, there is little or no due consideration to the substratum of cancer development. Sequel to some novel observations emanating from current understanding of cancer development, I reviewed the existing 10 hallmarks of cancer considering new biological trademarks that would augment further the understanding of tumor/cancer biology. I looked into the bedrock of tumor cells, considering that in line with the definition of current hallmarks of cancer, cancer cells may acquire all the functional capabilities to enforce its independent capacities and achieve autonomous potentials for proliferation, unending replication and subsequent metastasis, but without this adequate substratum obtained by colonization and stromatogenesis of the stroma, the development may not progress. I found that cancer development is highly based on the ability of the overwhelmingly mutated cells to colonize the normal tissue stroma in other to form their own stroma.

Keywords: tissue stroma, colonization, stroma- togenesis, hallmarks, cancer, tumor-+.

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ABSTRACT

Understanding the biology of cancer is one major advanced achievement in approach for cancer remission. Although discovery of 10 hallmarks of cancer has played a beneficial role towards this, guiding the understanding of cancer development, metastasis and drive to rational drug design, hallmarks of cancer may not have ended. Besides, there is little or no due consideration to the substratum of cancer development. Sequel to some novel observations emanating from current understanding of cancer development, I reviewed the existing 10 hallmarks of cancer considering new biological trademarks that would augment further the understanding of tumor/cancer biology. I looked into the bedrock of tumor cells, considering that in line with the definition of current hallmarks of cancer, cancer cells may acquire all the functional capabilities to enforce its independent capacities and achieve autonomous potentials for proliferation, unending replication and subsequent metastasis, but without this adequate substratum obtained by colonization and stromatogenesis of the stroma, the development may not progress. I found that cancer development is highly based on the ability of the overwhelmingly mutated cells to colonize the normal tissue stroma in other to form their own stroma. I highlighted on the strong defensive and accommodating framework of the normal stroma and its originality in body defence and revealed the: strong dependency of the aggressive mutated cells on the stromal framework; ability of the mutated cells to reverse the stroma activities for their independent development and subsequent invasiveness and metastasis, without which tumor microenvironment may not be formed. I suggested that stromal colonization and stromatogenesis is an acquired functional capability from mutational changes or genetic alterations that induced abnormal and aggressive behavior on the transformed cells, aggressive enough to overwhelm and polarize the defensive functional nature of the stroma cells to suit its autonomous progressive tendencies.

Questionable Issues at the back of this Review

- Considering the importance of tumour microenvironment, could establishment of tumour microenvironment be made possible without being aided and abetted by tissue stroma framework and the stroma cells sustainable activities?
- Hallmarks, in other words are trademarks of cancer cell which are inevitably identified with them. Considering the complexity of cancer cells and their biology of development, could there be further hallmarks of cancer in addition to the existing ones?
- Hallmarks of cancer has been described as fundamental functional capability of the cancer cells, can cancer cells gain these capabilities and utilize them without adequate tissue stroma as bedrock and engagement of stroma cells as aids?
- Research has shown that normal cells will not seed or survive an inappropriate micro-environment because they lack necessary cell autonomous survival signals. However, cancer cells undermine this local tissue hostility and evade anoikis during development and progression. The fact remains that this could be made possible because the tumor that could turn to cancer starts by interacting with the surrounding extracellular matrix (ECM), initiating a bidirectional relationship with its surrounding stroma.
- Why does it take **accumulation** but not singular cellular and gene mutational changes for cancer to develop in the surrounding tissues? Could it be that in the process of trying to colonize and exploit the stroma, it takes multiple or accumulated mutational

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changes to overwhelm the stroma due to its resistant nature and its functional ability to reestablish the homeostatic balance?.

Keywords: tissue stroma, colonization, stroma-togenesis, hallmarks, cancer, tumor-+.

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I. INTRODUCTION

Sequel to the tremendous efforts and the huge success trailing the explorations in the field of cancer which have resulted to wider understanding of its biology and developments, the hallmarks of cancer became manifest. The wonderful scientific work of Dauglas Hanahan and Bob Weinberg is highly appreciated in this direction. No doubt, the existing 10 hallmarks of cancer ealier proposed by [1, 2], currently are guiding the understanding of cancer development, metastasis and drive to rational drug design. However, hallmarks of cancer may not have ended. Leaning on the advancements and clearer understanding of different aspects of carcinogenesis in the recent years, doors are still open in search of more features to aid understanding of the disease and enabling approach toward diagnosis, prevention and treatment. In line with this, this review was embarked on, to develop further ideas towards clearer understanding of cancer/tumor development, invasiness and metastasis. inevitably, more efforts are required in other to achieve remarkable progress in cancer research; couple with some novel observations emanating from current understanding of cancer development, research is stirred to consider new biological trademarks that would augment the understanding of tumor/cancer biology, thus, I looked into the framework or the bedrock of tumor cells, with much focus on the foundational ground of tumor development considering that cancer cells may acquire all the capabilities to enforce its independent capacities and achieve autonomous potentials for proliferation, unending replicative potentials and subsequent metastasis, but without this adequate substratum, the development may be stalled. Cancer establishment is like someone who wants to have a progressive sleep; in this case, it is important to first secure a bed space before bringing in mat or bed. Sequel to the aforementioned axiom, formation of a clinically significant tumor may not be possible, without first engaging or colonizing a formidable surrounding normal tissue stroma irrespective of other capabilities possessed by cancer/tumor cells. It was emphasized in [3] that appreciation of tumor stroma is essential to an understanding of the biology of tumor growth. Besides, all solid tumors, regardless of their site of origin, require stroma if they are to grow beyond a minimal size of 1 to 2mm$^{4"}$ [3].

Hallmark of cancer was defined in [1], "as acquired functional capabilities that allow cancer cells to survive, proliferate and disseminate; these functions are acquired in different tumor types via distinct mechanisms and at various times during the course of multistep tumorigenesis" [2]. Subsequent to the initial six (6) hallmarks of cancer, many outstanding developments in cancer/tumor research have opened further observations, thus raising questions and ideas which encouraged revisit to the original hallmarks (The first Six Hallmarks) and ponder on others that are equally biologically involving. Therefore in 2011, Hanahan and Weinberg updated their hallmarks and came with four more hallmarks of cancer. This is evident that more hallmarks of cancer are inevitable and could continue to emanate considering the complexity of cancer. Hanahan and Weinberg publications on Hallmark of Cancer were influential reviews and highly relevant in the field of oncology. They compressed the developmental and metastatic knowledge of cancer biology into six major hallmarks and subsequently ten hallmarks including: "self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis" [2]; two enabling traits: "genome instability and mutation, and tumor-promoting inflammation" [2] and two emerging hallmarks: "reprogramming energy metabolism and evading immune response" [2]. Their reviews have become the backbone of

cancer biology and understanding of developmental and cancer metastatic traits. However, this great achievement by these two prominent great researchers is but not without criticism and challenges. Example, in [4], it was argued that cancer is a sequalae to malignant tumors and a hallmark, being a distinguishing feature, may not be peculiar to cancer but could be used to desdcribe tumor as well [4]. They alluded this to the fact that five of the six initial hallmarks (all except invasion and metastasis) are inherent in both benign and malignant growths and thus shows no distinctive value over non-malignant tumors [4]. Besides, nonmalignant conditions, such as endometriosis, have been reported to have their cells migrate to extra-anatomical sites and invade new tissues, yet maintain benign histological appearance [5]. Attempt to draw a more organized and updated picture of the cancer hallmarks was made in [6], when they defined seven hallmarks of cancer including: "selective growth and proliferative advantage, altered stress response favoring overall survival, vascularization, invasion and metastasis, metabolic rewiring, an abetting micro-environment and immune modulation". Their principle was based on "evolutionary perspective on the mutation theory in which carcinogenesis is a dynamic process that might initiate (and terminate) within cells, life-spans, with manifesting cancer hallmarks emerging throughout such a journey" [6]. The above were dusts reaised due to emergence of the work of Hanahan and Weinberg. Having said these, I found solace in the hallmark definition by Hanahan and Weinberg because I believe that no emerging unique hallmark of cancer would evolve without crossing or overlapping with the principles of their work. Trying to create entirely new basic hallmark principle, may lead to repetitions. However, I strongly uphold that there may not be a limit to derivation of new hallmark(s) of tumor/cancer or reviewing the existing ones until the disease is suppressed to a mere physiological headache that can easily be removed with paracetamol. This is based on the complexity, twisted and high levels of molecular interactions involved in the disease complex and considering the fact that environmental influences (exogenous and endogenous) could continue to add in manipulations of the disease process with regards to its complexity and kinks. The interest in this review however is to propose new hallmark in expansion of our knowledge about cancer, in line with its complexity and evolutionary trends, then augment the preventive and diagnostic measures and process of therapeutic drives.

Before moving further, it is important to understand the principles at the back of the mind of Hanahan and Weinberg that spurred the creative review that has brought progress in the field of research today. First, they viewed cancer research development as a rational scientific study, where the disease complications, described in the laboratory and clinic, will become understandable in terms fundamental values. They perceived these values as fundamental problems or changes that would in the first place induce or redirect normal cells transformation into cancer/tumor cells. Secondly, long termed research observations showed that virtually all types of human cancers have in common small number of molecular, biochemical, and cellular traits-acquired-capabilities [2]. They held strongly to an interpretation owing to the knowledge from the teachings of cell biology "that virtually all mammalian cells carry similar molecular machinery regulating their proliferation, differentiation, and death"[2]. Such similar molecular or cellular machineries constitute the bases of hallmarks of cancer. Thirdly: their reviews proved to them that the process of tumor/cancer development in human has multisteps echoing genetic mutations that initiate the progressive transformation of normal cells into highly malignant spinoffs [2]. Fourthly: "Many types of cancers are diagnosed in the human population with an age-dependent incidence implicating four to seven rate-limiting stochastic events" [2]. Fifthly: "Pathological analyses of a number of organ sites reveal lesions that appear to represent the intermediate steps in a process through which cells evolve progressively from normalcy via a series of pre-malignant states into invasive cancers" [2]. "Moreover these observations were concretized in [7], with indications that the genomes of tumor cells are

invariably altered at multiple site, having suffered disruption through lesions as subtle as point mutations and as obvious as changes in chromosome complement. With these at the back of Hanahan and Weinberg mind, it is evident that whatever could qualify as a hallmark of cancer, must be common traits in all cancers. Example, "virtually all mammalian cells carry similar molecular machinery regulating their proliferation, differentiation, and death" [1]. With regards to numerous distinct cancers and tumors that develop in a particular organ, sequel to abnormal cell regulatory circuits, the following questions also guarded their discovery of hallmarks of cancer: "How many distinct regulatory circuits within each type of target cell must be disrupted in order for such a cell to become cancerous"? "Does the same set of cellular regulatory circuits suffer disruption in the cells of the disparate neoplasms arising in the human body"? "Which of these circuits operate on a cell-autonomous basis, and which are coupled to the signals that cells receive from their surrounding microenvironment within a tissue"? "Can the large and diverse collection of cancer-associated genes be tied to the operations of a small group of regulatory circuits"? [1]. Based on the above considerations, it was suggested in [1] that the vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth, resulting to the six hallmarks of cancer development. "Each of these physiologic changes and novel capabilities acquired during tumor development represent the successful breaching of an anticancer defense mechanism hardwired into cells and tissues" [2]. Proposal that these six capabilities are shared in common by most and perhaps all types of human tumor was made in [1].

Other distinct attributes of tumor cells, proposed to be functionally important for the development of cancer and considered to be emerging hallmarks of cancer where reported by [8, 9, 10] and they include (1) major reprogramming of cellular energy metabolism in order to support continuous cell growth and proliferation, replacing the metabolic program that operates in most normal tissues and fuels the physiological operations of the associated cells and (2) active evasion by cancer cells from attack and elimination by immune cells; this capability highlights the dichotomous roles of an immune system that both antagonizes and enhances tumor development and progression. It is therefore critically evident that considering the complexity of cancer biology and the quest to untwist the foldings, more attributes that could be used to understudy tumor towards cancer development and subsequent metastasis must have emerged, thus the essence of this review. It is under these guides and guise that I propose Tissue Stroma Colonization and Stromatogenesis as additional hallmarks of cancer.

We consider tumor/cancer cells to be highly trickish, smart, sensitive, and dynamic with pragmatic evolutionary tendencies. Research has shown that under normal circumstances, developmental and survival specificity exist in environmental niches, under the biological control of microenvironment [11], indicating that normal cells do not venture or thrive in another environment else, the cell will encounter the hostility of tissue in form of detachment-induced cell death (anoikis) in that environment [12]. This simply means that normal cells will not seed or survive an inappropriate microenvironment because they lack necessary cell autonomous survival signals [11]. However, cancer cells undermine this local tissue hostility and evade anoikis during development and progression. This could be made possible because the tumor emanating to cancer starts by interacting with the surrounding extracellular matrix (ECM) [13], initiating a bidirectional relationship with its surrounding stroma [14, 15]. The tumor cells finally carve a niche from the stroma, different from the well organized and physiologically controlled normal tissue stroma. This is evident in reports from [16,15,17] emphasizing on initial engagement of the normal stroma by tumor cells, to enable further processes in development such as invasion and subsequent metastasis. This introduces the importance of cancer cells to first colonize and carve a niche in form of framework to avoid anoikis and to abet initiation and

progression. This is referred to in this review as tissue stromal colonization. Some of the cellular activities recorded during this stromal colonization, sequel to creation of tumor microenvironment (stromatogenesis), to sustain tumor progression include modifications of the extracellular matrix composition, activation of fibroblasts, myoepithelial cells and the recruitment of pericytes or smooth muscle cells, immune and inflammatory cells as indicated in [18].

As stated in [2], tumors exhibit another dimension of complexity as they contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the tumor microenvironment. The importance of this characteristic was initially recognized as enabling characteristic to generate the hallmarks traits, but the importance and process of acquisition of the normal tissue stroma as framework or bed of cancer/tumour cells to form the tumor microenvironment (tumor stroma) was not recommended amongst the existing hallmarks of cancer. Engagement or colonization of the stroma as a bed rock of the functional cells (the parenchyma cells), is therefore proposed here as the chief of the acquired functional capabilities by cancer cells to abet initiation and progression. Besides, development of tumor microenvironment depends on the initial engagement of the normal stroma (without which development of tumor microenvoronment could be stalled) which is made up of original standard cells functional in stromal tissue and subsequent tumor microenvironment [18].

So it is suggested here that tumor cells first hijack or colonize and devastate the normal stroma to create tumor microenvironment. This unique dynamic environment called tumor micro-environment could not have emerged without tumor interaction with the host [19] basically the host stroma. The interaction with the host stroma could be orchestrated by accumulated cellular and gene mutational changes taking place in surrounding tissues. The importance of tissue stroma and subsequent tumor stroma is so intriguing for its colonization efforts by tumor/cancer cells to be ignored as a hallmark of cancer. The development between stroma tissue and tumor microenvironment (tumor stroma) is like engagement of farmland and various nutrients in farm land to develop a fertile garden. The farm land is the stroma; the nutrients are the stroma cells while the garden is the tumor microenvironment. On the same farmland, the nutrients on the farmland could be used to create an orange orchard (the garden) thus tumor microenvironment is like a garden created out of a farmland and nourished by the nutrients of the farmland. This hypothesis is supported by the earlier pronouncement of [20], that "the constant bidirectional interaction of epithelial cancer cells with the surrounding microenvironment allows damaged stromal cells usage as a source of nutrients for cancer cells, maintains the stroma renewal thus resembling a wound that does not heal, and affects the characteristics of tumor mesenchymal stem/stromal cells (MSCs)". The normal stroma is the farm land with nutrients (stroma cells) upon which tumor micro-environment (garden) was made. The garden could not have been created without the farmland as the framework. The farmland could be engaged for creation of different types of garden depending on the choice of cultivation. Tumors "contain a stromal compartment (the garden), which is composed of stem cells, tumor-associated fibroblasts (TAFs), endothelial cells, immune cells, adipocytes, cytokines, and various types of macromolecules comprising the extracellular matrix (ECM)" (the nutrients) [20]. The normal stroma environment has its normal function until it is engaged by the tumor cells which have suffered accumulated genetic and epigenetic mutations and during which certain levels of cells polarization ensue. It is at this juncture that tumor microenvironment sets in and this is completely dependent on the fact that normal stromal tissue and the stroma cells are nutrient-like enough to form such a bed for tumorigenesis. The emphasis here is that colonization of normal tissue stroma is a prerequisite for tumorigenesis/carcinogenesis, thus a hallmark for tumorigenesis/carcinogenesis. In line with this, it was reported in [21] that "the interaction between stromal cells and tumor cells

(mutated cells) is known to play a major role in cancer growth and progression". The stromal cells have also been seen in bone marrow with special role in haematopoiesis and inflammations. In the skin, the growth factors released by the stromal cells mediate cell division [22], thus, enabling background regeration support of the epidermis from the bottom layer to replenish the sloughing cells at the top layer [22]. Moreover, the inflammatory role and mediation of cells accumulation to site of inflamed tissue as indicated in [22], is highly notable. Cancer/tumour cells do not operate in isolation, so it is a foremost character or feature of cancer/tumour cells to engage the tissue stroma and the stromal cells for oncogenic process prior to cancer development. Therefore engagement or colonization of normal tissue stroma and stromatogenesis is strongly regarded here as a forgotten, ignored and initially or previously unrecognized hallmark of cancer.

I consider the use of stroma by tumor/cancer cells to form their bed, as the chief hallmarks or strong trademarks of tumors/cancer because without the rocks on its bed, the stream may not have any song or noise. Suffice it to say that cancer/tumour without stroma is like garden without land or stream without ground. By the definition of hallmarks of cancer according to [1], the ability to engage/colonize and devastate normal stroma is a strong acquired functional capability for initiation, progression and even metastasis of cancer and therefore should be regarded as a hallmark of cancer. The subtopics presented below are analyzed with the intention to expose the possible oncogenic potential factors as envisiaged in their normal physiological functions.

II. THE TISSUE STROMA

Even though tissue epithelium is made up of highly specialized cells that orchestrate specific activities, the proper development and function of the cells are not without contextual signals from the stroma [23, 24]. The stroma referred to as layer or bed, "is the part of a tissue or organ with a structural or connective role". As indicated in [25], it is made up of all the parts without specific functions of the organ - for example, connective tissue, blood vessels, nerves, ducts etc, while the other part, the parenchyma, consists of the cells that perform the function of the tissue or organ. Furthermore, stroma is composed of stromal cells which may be indirectly involved in hematopoiesis, providing a microenvironment that influences the function and differentiation of hematopoeietic cells enabled by colony stimulating factors, which they generate [25]. Cell types that constitute the bone marrow stroma include: fibroblasts (reticular connective tissue) macrophages, adipocytes (fat cells) osteoblasts (synthesize bone) osteoclasts (resorb bone) endothelial cells, which form the sinusoids [26].

Crosstalk between the emanating tumor cells and the creation of tumor stroma, highly responsible for the progression of tumors and their metastasis has been increasingly unveiled [27, 28]. As such, a lot of importance has been attached to tissue stroma. Crosstalk between the host stroma involvement in carcinogenesis should be seen as a strong panacea or enabling link to important signaling pathways promoting growth, considering that cancer is a disease of signaling malfunction in which case signals to growth-inhibiting genes (tumor suppressor) are disabled [29] and rather abets the activation of a growth-promoting (oncogene) pathway by genetic mutation which could lead to novel therapeutic interventions targeting tumor stroma receptors [29]. "Stromal connective tissues are found in the stroma and belong to the group of connective tissue proper". "The function of connective tissue proper is to secure the parenchymal tissue, including blood vessels and nerves of the stroma, and to construct organs and spread mechanical tension to reduce localized stress" [30]. Furthermore, "stromal tissue is primarily made of extracellular matrix containing connective tissue cells and extracellular matrix is primarily composed of ground substance-a porous, hydrated gel, made mainly from proteoglycan aggregates - and connective tissue fibers" [30]. The stromal cells are endowed with the capacity of becoming the connective tissue cells of many organs of the body. Basically, in [31], normal stroma was reffered to as a "Native Micro-

environment (NME) of live tissue and described it as a "mechano-physiological space provided to tissue cells, which in turn contribute to the overall appearance and function of the tissue", indicating that "NME is rather specified on the basis of physical, physiological, metabolic and other functions of particular tissues or organs" [31]. It has been demonstrated in [32, 31] that, the bone microenvironment is necessary for normal growth of bone tissues while the heart microenvironment is essential for cardiomyocytes, other heart cells and blood vessels are to maintain the heart muscle kinetic functions [32, 31]. Normal stroma " therefore plays vital roles in maintaining the integrity and functionality of tissues ranging from growth and static to kinetic activities, with an exception in regenerative microenvironment (RME), where a reprogrammed tissue growth is involved" [31]. This is evident to the capital reason why developing tumor cells colonize tissue stroma to enable progression. This is also evident to say that tumor stroma (tumor microenvironment) is not a normal physiological niche like the normal tissue stroma but a pathological niche influentially created by persistent genetic and epigenetic instabilities. The current dominant paradigm with the impression that multiple genetic and epigenetic "lesions provide both the impetus for and the Achilles heel of cancer development, might be inadequate to understand cancer as a disease process" [33], without the enabling framework, the tissue stroma. Therefore the undertakens between tumor cells and the tissue stroma needs to be well understood. In support of this, reports earlier described "Tumor microenvironment (TME) as an abnormal native physiological condition, where tumor cells and their associated stromal cells undergo uncontrolled growth, proliferation, migration, excessive deposition of certain extracellular proteins and other cancerous cellular activities that result in irregular ECM networks and tissue growth" [34, 2]. Therefore, I uphold here and in line with the report of [35], that the accumulated genetic alteration would lead to abnormal cell growth and subsequent malignant cells. Persistence of these genetic changes consequently changes the normal defensive stucture of the host stroma compartment to enable a permissive and supportive environment for invasive tumor/cancer cells. This is the engagement and activation of the normal tissue stroma which could be regarded as the first line of the process after multiple genetic alterations and development of malignant cells. The evolutionary trend is established by accumulation of genetic and epigenetic irrationalities which impact negatively on their surrounding environment, recruiting and corrupting non-malignant cells in the area thus form what is known as a tumor microenvironment (TME) [36, 31, 37]. What forms or induces tumor microenvironment is naturally, normally and functionally in existence in the body, yet the body is devoid of tumour microenvironment until the normal and functional stroma is adversely engaged and devastated to create tumor microenvironment. Suffice it to say that tumor microenvironment is an oncophysiological tissue space initiated by tumorigenesis with enabling framework the stroma. Tumor microenvronment is a trademark of both solid and liquid tumors, thus virtually all cancer development are initiated by engagement of normal tissue stroma and "require stroma for nutritional support and for the removal of waste products" [38]. "In the case of leukemias, blood plasma serves as stroma" [38], "although an additional stromal response, angiogenesis, may develop in the bone marrow" [39]. Tumors growing in the body cavities have plasma exudates (ascites) as their stroma [40]. Where solid tumors are involved, connective tissues, blood vessels, inflammatory cells, serve as stroma, interposing between the malignant cells and normal host tissues. In all tumors, stroma is largely a product or an averse donation by the host and is induced as a result of tumor cell-host interactions, while in tumors of epithelial cell origin (carcinomas) a basement membrane is often interposed between the tumor cells and the stroma, but in other types of tumors, malignant cells directly abut on or intermingle with stromal elements" [41]. These reports backup my indications that where there is no capability for stroma colonization, no tumorigenesis or carcinogenesis.

Normal tissue stroma could also be referred to as source of maintenance of normal body

homeostasis which alteration by tumor cells could be seen as a hallmark of cancer development. This could as well be regarded as alteration of normal body homeostasis to generate homeostatic instability. Therefore alterations of the normal tissue stroma would generate homeostatic instability that induces cell polarity to favor tumorigenesis and even cancer metastasis, which aligns with explanation on homeostasis in [42] as a general principle that safeguards the stability of natural and artificial systems, where stability is understood in its more classical sense of robustness against external perturbations.

2.1 Stromal cells and their Bionatural Potential Oncogenic Factors

Presention of stromal cells as essential component of the stromal scaffold of the bone marrow that provides physical and functional support during hematopoiesis was made in [43]. It was further indicated in [43], that stromal cells engrafted into immunodeficient non-obese diabetic severe combined immunodeficient (NOD-SCID) mice give rise to pericytes, myofibroblasts, bone marrow (BM) stromal cells, osteocytes, osteoblasts, and various endothelial cells. Stromal cells also reffered to as Mesenchymal Stromal cells (MSCs), is one of the most studied types of stromal cells as indicated by [44] and [45] and are further shown to be a heterogeneous population of adherent cells with fibroblast morphology that proliferate in vitro and can differentiate into osteoblasts, chondrocytes, and adipocytes [43]. MSC acronym used for Mesenchymal stromal cells here should not be confused with mesenchymal stem cells. To address this inconsistency between nomenclature and biologic properties, and to clarify the terminology report in [46], it is suggested that the fibroblast-like plastic-adherent cells, regardless of the tissue from which they are isolated, be termed multipotent mesenchymal stromal cells, while the term mesenchymal stem cells is used only for cells that meet specified stem cell criteria [46]. Researchers have defined mesenchymal stromal cells (MSCs) as multipotent cells that are able to adhere to plastic surfaces, have the capacity to differentiate into osteoblasts, chondrocytes or adipocytes in culture, and express the cell surface markers CD73, CD90, and CD105, but not any leukocyte markers [47, 48, 49], while conventional stem cell properties such as plastic adherence and the expression of CD44, CD90 and CD105 are unspecific for stem cells [47, 48, 49]. Moreover, both fibroblasts and MSCs have been shown to have multipotent and immuno- modulatory functions [50]. Comparative efforts to separate the two cells (MCSs and fibroblast) and also indicate similarities that colony-forming capacity and differentiation potential are specific important properties that discriminate MSCs from fibroblasts was made in [48]. Additionally, while both MSCs and fibroblasts occupy the stroma of many tissues, MSCs are capable of migrating through the body via blood vessels, whereas less evidence exists that fibroblasts migrate via circulation [51]. Relative to fibroblasts and other stromal cell types, the primary functions of MSCs are to regulate the immune response and to promote tissue regeneration and are also the sources of osteoblasts and chondrocytes in bones and joints respectively [52, 53]. In the cause of this review, the acronym MSC is used for mesenchymal stromal cells. The International Society for Cellular Therapy (ISCT) encourages that the scientific community or investigators must clearly define the more scientifically correct designation in their reports adopt this uniform nomenclature in all written and oral communications [54].

Research has indicated that mesenchymal stromal cells (MSCs) possess self-renewal capacity and multilineage differentiation potential, indicating their prospects as cellular therapeutic agents for regenerative medicine [55]. These cells have also been shown to be suitable for use in clinical applications because of their various properties, such as: low immunogenicity, immunomodulatory effects [56]; and migration potential to sites of injury and regenerative potential [57]. The Mesenchyme which is the source of mesenchymal stromal cells is the first embryonic mesenchymal tissue to emerge, and it is produced from epithelial mesenchymal transmission (EMT) in epiblast cells [57]. It is an unspecialized cell which occurs during gastrulation which is the process in which the three primary germ layers, endoderm, mesoderm, and ectoderm develop

during the embryonic development of an animal" [58]. "It is induced by the primitive streak through Wnt signaling, and produces endoderm and mesoderm from a transitory tissue called mesendoderm during the process of the gastrulation [58].

III. STROMA COLONIZATION AND STROMATOGENESIS

Ealier research has shown that cells in the nonmalignant stroma (normal tissue stroma) are usually in a quiescent state and maintain homeostasis in the ECM and epithelial compartment, in part by negatively regulating the proliferation, motility, and invasion of cells in the epithelial layer [49, 31]. As early as 1989, it was reported in [59] that the majority of neoplasms, as well as healthy viscera, are composed of the parenchyma and nourishing or supporting tissues, comprising the stroma [59]. It was indicated that tissue stroma generally do not take part in neoplastic transformation and are comparable to other components of the host which are not neoplastic, however, the tissue stroma is directed by the neoplasms, at least partially during the neoplastic disease process [59]. Sequel to this, I suggest that the tumor stroma and the accompanying cells could not have been contracted ex-vivo but from within the developmental niche. Therefore, I uphold that the molecular composition of the host stroma is different from that of the tumor stroma as reported by [60, 49]. However it is important to note that this difference could be as a result of the persistence or chronic inflammatory engagements on the host stroma which must have activated lots of molecular activities such as cytokines, chemokine and fibroblast to mediate cellular and molecular polarization in the host stroma, this polarization therefore, enables acquisition of protumor potentials by the cells and thus lose their host defensive potentials. This phenomenon is capable of imposing distinctive molecular expression status to tumor engaged stroma. This submeission, is in line with the emphasis laid by many research works in [61, 62, 60], that the MSCs and cancer associated fibroblasts (CAFs) localized in the tumor stroma have a different phenotype compared to MSCs and fibroblasts isolated from normal tissues, which may be as a result of the constant exposure of these cells to inflammatory and cancer cell-secreted cytokines inducing procancerous characteristics. Before now, transformation of the stroma to desmoplastic loosely vascularized dense connective tissue and the remodeling of the stromal extracellular matrix (ECM), inducing local cancer cell migration and metastasis have been indicated in [63, 64, 65 and 66]. Other reported gradual evolution process of the tumor microenvironment include: increased local vascular permeability; the extravasation of plasma and macromolecules, such as fibrinogen and plasminogen; the activation of coagulation mechanisms in the developing tumor microenvironment; the formation of fibrin gel deposits; the formation of a provisional stroma comprising cancer cells, fibroblasts, and immune cells; the initiation of angiogenesis in the provisional stroma; the degradation and replacement of the provisional stroma fibrin with highly vascularized granulation connective tissue as described in [63, 64, 65 and 66]. It has earlier been reported in [67, 68, and 20] that tumors are organ-like structures, composed of numerous cell types whose interactions are required to drive and promote their growth and metastasis.

To substantiate the claim on stromal colonization by transformed or highly mutated cells and strongly support its importance as a highly significant omen or event in tumorigenesis or carcinogenesis, I lay credence to the reports from the work in [68], titled "changes in the tissue surrounding a growing tumor and the significance of the precancerous state". Here, the results of the experimental investigations on the titled above were summarized under the indication that the changes so frequently observed in tissues surrounding a growing tumor may be caused by different conditions: (1) the development and growth of a malignant tumor depends upon a local interaction between tumor cells and organ cells, when the cells of a normal organ are capable of inhibiting tumor growth, then an impairment of the normal state of the parenchymatous cells of this particular organ is essential" [68]. He posited

that this precancerous state does not consist primarily of an inflammatory change in the adjacent connective tissue, as indicated in [67]. (2) in a degeneration of the parenchymatous cells of the host organ, if, in another instance, the cells of the normal organs are unable to inhibit the proliferation of the tumor cells, then no preparation of the cells of the organ is necessary for the tumor to grow, i.e., no precancerous state is needed to enable the tumor to grow. On the other hand, the proliferating tumor cells injure normal cells, either mechanically or chemically, producing a condition that appears on superficial examination like that described as the precancerous state. In reality, however, this condition is the resultant effect of the tumor growth and may be more correctly designated the postcancerous state. Still, of greater importance is the submission in [68] that the general condition of resistance or immunity to cancer growth exerts a greater influence on the organism of the animal than any of the local conditions described above. The local resistance of a testicle to tumor growth in a generally susceptible animal may be overcome, but if an animal is made generally immune to the growth of cancer, neither the animal as a whole nor a single organ or tissue in it can be made susceptible to the growth of the tumor [68]. With this, it shows that the importance of tissue stromal colonization by cancer cells has been on debate long time ago.

My evocation on this is that looking at the Levin's submissions in [68] emanating from original research works performed to determine the actual event taking place at the incipient of cancer establishment on a tissue or organ, from the current perspective, this is turned to mean that inflammation does not just occur without irritants and as far as the defensive mechanism of the organ can surmount the irritants, the ensuing inflammation runs on acute state and resolves after the irritants have been removed. But if the host is short of defensive efforts and the irritants persist, it will eventually overwhelm the host. Now in the case of cancer cells, these cells experience multiple genetic and epigenetic alterations. This is in line with the report of [69], that advances in gene expression, genetic, and epigenetic profiling of stromal cells have improved our understanding of how mesenchymal-epithelial cell interactions may create a permissive microenvironment for malignancy and identified potential targets for cancer prevention and treatment including chemokine and cytokine networks. Furthermore, it was determined in [70], that extensive gene expression changes occur in all cell types during cancer progression and that a significant fraction of altered genes encode secreted proteins and receptors. Importtantly, it should be noted that despite the dramatic gene expression changes in all cell types, genetic alterations were detected only in cancer epithelial cells by [69], indicating that tumor only manifests when genetic alterations must have overwhelmed the stroma and the cells. Many works have emphasized that reciprocal interactions between responding normal cells, their mediators, structural components of extracellular matrix (ECM) and genetically altered neoplastic cells, regulate all aspects of tumorgenicity. Furthermore, CXCL14 and CXCL12 chemokines overexpressed in tumor myoepithelial cells and myofibroblasts respectively, bind to receptors on epithelial cells and enhance their proliferation, migration, and invasion [71, 72, 73, 18]. These informed the source of the initial changes in the stroma followed by the gradual overwhelming suppressive activities being mediated by the mutated transformed cells. Possibly, at the initial stage of the alterations, may be at the stage of only one mutation, the host immune defense continues to resist the neoantigens and at this stage, cancer or tumor growth may not develop unless there are external influences against the tissue, like chemical toxins to suppress the tissue parenchymatous cells (considering levin's first submission). But as the cells continue to experience gene mutations, even the immune stability in the host could lose defense due to persistent generation of the neo antigens resulting from multiple and overwhelming gene alterations. Suffice it to say that it may not necessarily be that the host is originally immuno-compromised but that the surrounding cells have suffered numerous gene mutations that could resist the immune response. So when this happens, the transformed cancer cells can override the activities of the

parenchymal cells without additional aid (as indicated in Levin's second submission). Yes, inflammation may not play a passive role at the stage when only one mutation has occurred instead it will play an acute tissue defensive role to protect the environment. But at the stage of multiple overwhelming mutations, the transformed cancer cells will injure the normal cells by biochemical secretions and damages thus suppressing the surrounding or connective tissue cells function, activating the usual quiescent cells and subsequent emanation of their phenotypes, attracting and polarizing immune cell and subsequent secretion of cytokines and chemokines such as cytokine release symdrome (CRS), as well as overwhelming level of immune complexes to mediate protumor effects. Suffice it to say that a lot of unnoticed or asymptomatic events have been going on in the cancer possessed tissue possibly years long before the tissue is overcomed by the cancer cells and exhibit symptoms. At the stage in which the cancer cells have overcome the tissue cells, Levin indicated that no precancerous state needs to be induced. In line with the submissions of Levin, inflammation may not induce or aid cancer growth when the cells have not suffered enough mutation to sustain chronic inflammation as against the claims in [67], but when the smoldering inflammation is sustained by multiple mutations to persist and develop into chronic inflammation, tumor growth may ensue if no counter measures are taken. So this is the initial step in cancer development meaning that it takes multiple genetic and epigenetic mutations to colonize the tissue stroma and redirect the formal homeostatic, tissue defensive nature of the stroma to favor tumor growth. By doing this, the cancer cells carve a niche from the stroma to avoid anoikis as stated above which thereafter known now as tumor stroma. It is not therefore scientifically and clinically proper to jump or ignore this initial capability of cancer cells, the basic effort by the cancer cells to initiate and progress amongst the halls of characteristics or trademarks of cancer, referred to as hallmarks of cancer. Meanwhile this understanding, may reveal the possibility of stopping cancer at the earliest stage of development but however demands for regular check up and adequate laboratory analysis to achieve this. Research has shown in [74] that most transformed cell lines are not able to survive after transplantation and are therefore considered to be cells continuously growing without enough tumorigenic potency (more acquired capabilities needed), even among highly carcinogenic cell lines, only a small subset harboring stem cell-like characteristics are able to initiate tumor growth *in vivo* [74]. This indicates that, either the cell line could not engage adequate stroma or that the host tissue stroma is immune-competent enough to resist the initiation of the cancer, which is reffered to as 'elimination' in tumor immune response interaction. In other words creation of tumor microenvironment is not innate or constitutive, but requires functional capability of the cancer development process to colonize and devastate the host stroma prior to progression.

It is obvious that the transformed (carcinogenic) cells, recruit nontumorigenic cells both locally from the neighboring tissues as well as from the circulation to enable the tumor micro-environment, sequel to this, it is understandable from the submissions in [75, 76], that through reciprocal cancer-stroma interactions, tumor microenvironment co-evolves to promote cancer progression through paracrine signaling and physical interactions. It is evident in [61, 60, 49], that when cancer develops, the stroma undergoes vast changes to become fibrotic and activated, the ECM becomes denser and more rigid, and is composed of alternative forms of connective fibres, such as tenascin and fibronectin, which cancer cells can invade. Fibroblasts and MSCs change shape and expression profiles and become more proliferative and secrete higher levels of growth factors, cytokines, and chemokines. These changes or polarizations result to stromal phenotypic cells operating in the tumor stroma. Evident is on the fact that the normal tissue cellular composition is the same with the tumor stroma cellular composition as indicated overleaf, but differ in molecular composition. It is also in evident [68], that the tumor microenvironment also contains cancer-associated fibroblasts (CAFs), endothelial cells [77, 78], immune cells [79, 80], adipocytes [81], cancer stem cells (CSC)

that differentiate into metastatic epithelial cells [82, 83], mesenchymal stromal cells (MSCs) that can differentiate into fibroblasts and other types of cells representing mesenchymal lineages [84] and various types of extracellular matrix (ECM) proteins [68], needed for reciprocal messaging and the stimulation of tumor growth. Research has earlier shown in [75], that the stroma and stromal cells originating from MSCs, have been recognized as players in carcinogenesis, affecting tumor growth, development, and progression beginning at the early steps of tumorigenesis, influencing the construction of the micro-environment, epithelial mesenchymal transition, and metastasis, that is, functions that are essential for tumor maintenance and metastasis to other tissues [85, 86].

Tissue stroma is regarded in this study as a bed or farmland or ranch, framework, "a fundamental feature of the architecture and functional design of vertebrate animals is a stroma, composed of extracellular matrix and mesenchymal cells, which provides a structural scaffold and conduit for blood and lymphatic vessels, nerves, and leukocytes [87]. The stroma cells, embedded on the stroma include the mesenchymal cells and epithelial cells as mentioned above [87]. Colonization or engagement of these structural scaffold and conduit and its cellular components by the cancer cells initiates strotomagenesis (tumor stroma). Stromatogenesis therefore is the formation of new, specific type stroma at sites of active tumor cell invasion as an integral part of the invading process [88], just like a garden carved out from the farmland as described above. During tumorigenesis, reciprocal changes in stromal fibroblasts and tumor cells induce changes to the neoplastic microenvironmental landscape [89]. In stromagenesis, both the complex network of bi-directional stromal fibroblastic signaling pathways and the stromal extracellular matrix are modified [89]. The parenchyma of a carcinoma is composed of those cells from either an epithelial lining membrane or a gland, which underwent malignant transformation. In contrast, the stroma of such a neoplasm, composed of the original stroma of the affected organ or of the newly initiated stroma of the tumor, is non-neoplastic in character [89]. This is supported by the findings in [90], that the stroma as colonized by the cancer cells has no mutational changes unlike the cancer cells which have suffered lots of mutational changes to enable transformation. It is also evident that the unmutated host tissue stroma was colonized by the mutated cancer cells to gain a soft landing, with emphasis on the different mutational status [90]. "Enzymatically it relates to the control of the neoplastic parenchyma to a greater or lesser degree. The stroma plays a definite role in different stages of tumor development beginning with secretion of angiogenetic factors and other starting tumor developments of which the progression of the tumor depends on the stromal reaction"[59].

The newly formed tumor stroma was mentioned by [88] as engaging a void space, i.e. at the free surfaces, whether internal or external (extramural stromatogenesis for tumors) and that the new stroma formation is generated and governed by the invading tumor cells with the tolerance and complicity of the adjacent activated fibroblasts [88]. The "seed and soil" hypothesis was presented more than a century ago by [91, 92], but until recently, we began to understand the complex crosstalk between the tumor cells ("the seeds") and the tumor-growing microenvironment ("the soil"). We opined that this soil occupies the farmland (the tissue stroma) which tumor cells have smartly spotted, fertilized and colonized to form a bed for growth and progression. This is an indication that the tumor microenvironment (the soil emanated from the farmland (the stroma), both serving as base required for tumor growth, enabling framework for proper nurturing. Soil should not be mistaken as land, soil is rather embaded on the land and could be washed off by any enabling force such wind or flood while the land remains on point. Lands are different from soils, this while soils on lands may differ. By this illustration, tumour microenvironment (soil) could be separated from the stroma (land). The tumor growth or development is highly dependent on these compartments. This is sequel to the report that interactions between cancer cells and the stromal compartment have major impacts on

cancer growth and progression [93]. Earlier, on this issue of seed and soil concept in [94], it was indicated that cancer cells, called seeds, survive in a highly complex microenvironment of the surrounding stroma, called soil. Describtion in [88] showed that the new stroma (tumor stroma) is neostroma and "that the neostroma is complementary to cancer cell metabolic activity, important for buffering of cancer cell waste products and for the prevention of cancer cell acidic death. Thus, cancer cells and neostroma should not be seen as a mixture of heterogeneous uncoordinated cells but rather as a unified morphologic and metabolic domain with a harmonious collaboration between aerobic (myofibroblasts, endothelial cells) and anaerobic compartments (cancer cells) as indicated in [88]. In line with these, it is hypothesized in this research that Cancer without stroma is like faetus without womb/placenta or chick embryo without egg sac. Tumor inducing genes are normally and functionally in existence in the body but the body is devoid of tumor until the genes are mutated and tumor evolutionary trend established. This means that tissue stroma is normally in existence with its normal physiological functions until it is colonized by transformed tumor cells and subsequently activated. Probably, these above avowals would drive home the importance of this forgotten and deemphasized hallmark of cancer being proposed in this article. This informs the reason why [95], indicated that "a tumor cannot develop without the parallel expansion of a tumor stroma". Meanwhile, one of the histological cornerstones of cancer development is the formation of a dense fibrotic stromal matrix comprising ECM and activated fibroblasts (myofibroblasts). In this last phase of stromal development, the granulation tissue transforms into desmoplastic dense connective tissue characterized by poor vascularization [95], additionally in [96], it was mentioned that the expansion of the tumor stroma with a proliferation of fibroblasts and dense deposition of ECM is termed a desmoplastic reaction, morphologically termed desmoplasia and initially intended for defense against tumor growth. This is secondary to malignant growth and can be separated from alveolar collapse, which shows neither activated fibroblasts nor the dense collagen/ECM [96, 97, 98]. On the other hand, data have shown that in established tumors, this processes are seen in several aspects of tumor progression, such as angiogenesis, migration, invasion, and metastasis [99]. It could be said that the activation of the stroma, desmoplasia, is an attempt by the tumor tissue to heal the injury produced by the infiltrative and destructive growth of cancer cells, indicating the invasive and malignant (colonization/stromatogenesis) charac-characteristics of the tumor. However, it has been suggested that the increased collagen synthesis in desmoplasia, together with myofibroblast-induced tissue retraction, may paradoxically constitute a protective mechanism with invasive character-istics [78].

Another important illustration is the similarity between stroma from wound and that from tumors. Though active angiogenesis and numerous proliferating fibroblasts secreting a complex ECM are seen in both stromas with ensuing background of fibrin deposition, the tumor stroma was notably referred to as activated or reactive stroma [3]. Evident of normal stroma colonization, indicating that "aggressive malignant cells are clever at exploiting the stromal environment, example, tumor cells can reside in the stroma and transform it" [100] and "modify the metabolism of resident cells" [101], thus tumor yield a stroma that is permissive rather than defensive (Normal tissue stroma) [27]. Cancer target therapies would be more successful if the crosstalk between normal stroma and tumor are adequately deduced, in line with understanding cancer genetics per se [27]. The stroma is determinant for the tumor progression and therefore is an important therapeutic target [102], thus its colonization is an omitted chief hallmark of cancer.

IV. STROMA TISSUE/CELLS IN CANCER DEVELOPMENT

As stated above, Tumor or Cancer microenvironment is not simply made of self-sufficient neoplastic cells but also composed of fibroblasts, immune cells, endothelial cells, and

specialized mesenchymal cells [27]. Originally, the cell types exhibit defensive function with their natural tumor-suppressive abilities and homeostatic responses in tissues before being unfortunately colonized, undergo alteration and transformed by the tumorigenesis or stromatogenesis and thus activated to support tumor growth and facilitate metastatic dissemination [27]. It should be noted as in [99, 49], that in tissue homeostasis, normal fibroblasts are in an inactive quiescent state, embedded within the fibrillar ECM primarily of collagen type 1, laminin, fibronectin, and proteoglucan and interact with their surroundings through cell receptors called integrins. "Fibroblasts become activated in wound healing and fibrosis", to give rise to these cells called myofibroblast [103]. It has been indicated that myofibroblast differ morphologically and functionally from quiescent fibroblasts and on activation, these cells are capable of producing relevant signal mediators, such as growth factors, cytokines, chemokines and other immune modulators [104, 27]. But as soon as the wound healing is completed, most of these activated fibroblasts are removed from the granulation tissue by apoptosis [105, 106]. This is evident that recruited tumor fibroblasts are originally in isolation with tumor micro- environment and that tumor microenvironment forms only when the tumor engages the stromal tissue and activates the stromal cells. Sequel to this, cancer has been considered a wound, however reffered to a wound that never heals because the activated fibroblasts are not removed by apoptosis as in normal wound healing [107, 108], thus indicating that cancer is a continued or persistent lesion that should have been eliminated by the acute stage of inflammation but rather the inflammatory response was overwhelmed by more radically transformed cancer cells with features that mediate polarization of or retention of activated stroma fibroblast, thus giving rise to a phenotype called cancer associated fibroblast (CAF), secretion of cytokines and chemokines inducing cytokine release symdrome (CRS) and migration of immune modulators to maintain the wound like environment and thus enabling immune evasion. So instead of the wound healing, these cells are rather prominent contributors in carcinogenesis [109], engaging the original mesenchymal and epithelial reciprocal interactions in morphogenesis of tissues and organs [87]. This is a tumorigenic ability. "The spindle cells of the 'neostroma' (Tumor stroma) are intensely proliferating myofibroblasts, which are characterized by the frequent expression of α-smooth muscle actin and the particularly frequent expression of thymidine phosphorylase, PDGF-receptors and SPARC (secreted protein acidic rich in cysteine)" [88]. This also is a copy of normal tissue stromal fibroblast.

The original defensive role of normal stroma fibroblast has been accessed with different research approaches including creation of a microenvironment that shares some of the features of tumor-associated stroma such as: regenerating myealinated fibres (RMFs) overexpressing hepatocyte growth factor (HGF) or TGFβ, alone or together, were used to humanize cleared fat pads prior to the introduction of breast organoids as stated in [110]; allowed for only normal outgrowths, the growth factor enriched RMFs allowed for the rare promotion of ductal carcinoma *in situ* (DCIS)-like lesions, adenomas and poorly differentiated tumors from ostensibly normal organoids unlike non-immortalized, normal primary human mammary fibroblasts [110]; tumors were efficiently generated from tissue recombinants when genetically modified organoids were co-mixed with immortalized fibroblasts with or without expression of HGF, showing that tumor development was rarely observed when organoids were implanted either alone or co-mixed with normal primary fibroblasts further demonstrating that human breast cancer formation, even in the presence of oncogene-driving mutations, requires activated stoma [111]. The above results further underscore the notion that "even in the presence of robust oncogene signaling, activation of the stromal environment is an important component for malignant transformation of human breast epithelium *in vivo*" [112]. These are substantial laboratory proven facts highlighting the importance of stroma and its colonization prior to tumorigenesis/carcinogenesis. "In tumors, activated fibroblasts are termed as peritumoral fibroblasts or carcinoma-associated fibroblasts

(CAFs)". "Stromal changes at the invasion front include the appearance of CAFs. Carcinoma - associated fibroblasts, constitute a major portion of the reactive tumor stroma and play a crucial role in tumor progression" [27]. In response to tumor growth, [27] showed that fibroblasts are activated mainly by TGF-β, chemokines such as monocyte chemotactic protein 1 and ECM-degrading agents such as matrix metall-oproteinases (MMPs) [27]. "Cancer associated fibtroblasts (CAFs) promote malignant growth, angiogenesis, malignant invasion and progression including metastasis" [113, 114, 115]. The roles of CAFs and their potential as targets for cancer therapy have been studied in xenograft models, and evidence from translational studies has revealed a prognostic significance of CAFs in several carcinoma types [116]. The trans-differentiation of fibroblasts to CAFs has been reported in [27] to be driven to a great extent by cancer-derived cytokines such as transforming growth factor-β (TGF-β), during which the normal stroma must have been engaged by the cancer/tumor cells. Under normal conditions, this TGF-β acts as a tumor suppressor by inhibiting proliferation, as previous studies have shown in prostate epithelial cell lines [117]. However, TGF-β has been shown to assume a protumorigenic role as cancer progresses [117]. In a study using an orthotopic xenograft model to reconstruct human mammary gland, results indicated in [111, 112], that overexpressing TGF-β in mouse fibroblasts could induce the initiation of breast cancer from normal epithelial tissue. TGF-beta causes cancer progression through paracrine and autocrine effects. Paracrine effects of TGF-beta implicate stimulation of angiogenesis, escape from immunosurveillance and recruitment of myofibroblasts, Autocrine effects of TGF-beta in cancer cells with a functional TGF-beta receptor complex may be caused by a convergence between TGF-beta signalling and beta-catenin or activating Ras mutations as shown in [118] where experimental and clinical observations also indicated that myofibroblasts produce pro-invasive signals and that such signals may also be implicated in cancer pain [118]. Similarly, N-Cadherin which is expressed in invasive cancer cells and in host cells such as myofibroblasts, neurons, smooth muscle cells, and endothelial cells, act as invasion-promoters, this has been shown in [118] to promote matrix invasion, perineural invasion, muscular invasion, and transendothelial migration due to N-Cadherin-dependent heterotypic contacts.

The foggy controversial role of normal fibroblasts in promoting cancer, has been made clearer by the studies in [119], which provided evidence that both normal and activated cancer-associated fibroblasts (CAFs) can promote breast cancer cell growth in vitro and in animal model systems, based on constitutively secreted cytokines, such as CCL7, IL-6, and IL-8, that can activate the release of platelet-derived growth factor BB (PDGF-BB) from breast cancer cells that stimulates release of IL1-β by the fibroblasts and in turn induces breast cancer cell proliferation [119]. Interestingly, in another study, fibroblast-derived CCL2 was shown in [120] to play a key role in promoting Breast cancer stem cells (bCSC), self-renewal and tumorigenesis in a Notch1-dependent manner both in vitro and in vivo. Moreover, CAF-secreted prostaglandins have been shown to promote secretion of IL-6 that results in bCSC expansion [121, 122]. Additionally, senescent primary normal breast luminal cells activate breast stromal fibroblasts in an IL-8-STAT3 pathway-dependent manner [123]. These activated fibroblasts displayed pro-carcinogenic features and promote a CSC-like phenotype by increasing expression of stem cell markers, such as CD44, ALDH, SOX2, OCT4, NANOG, and KLF4 and also induced epithelial mesenchymal transition (EMT) in breast cancer cells both in vitro and in vivo [123]. "Earlier study in [124], demonstrated that Sonic Hedgehog ligand secreted by TNBC cells confers and activates normal stromal fibroblasts. These activated fibroblasts in turn secreted FGF5 and produce fibrillar collagen-rich ECM essential for maintenance of the CSC phenotype and development of chemoresistance [124]. Another study in [125] showed that breast cancer cells activate fibroblasts and induced secretion of chemokine ligand 2 (CCL2). These wide research largely elicit and implicate stromal fibroblasts activities especially their activated derivatives in

stromatogenesis and tumorigenesis/carcinogenesis.

As earlier stated, the working model in the field (microenvironment) is that typically when we think about the primary tumor, the normal stroma being engaged or under devastation is primarily preventing tumor growth. This is both through normal fibroblasts, as well as through the immune system, which surveys our body to prevent the outgrowth of abnormal cells. But importantly, as indicated by [61], understanding the cross-talk between the host stroma, the stromal cells and tumor cells is highly inevitable in the quest to appreciate process of tumor growth and progression. This is because as the situation persists and progress, many of these anti-tumor components are lost owing to polarization due to chronic inflammation being fueled by persistent cytokines and chemokine activities as indicated in [61, 60]. This may possibly include accumulated immune complexes. Indicating that, there are many pro tumor components actually suppressing the activities of normal tissue cells in the in-situ formed tumor microenvironment. Due to their phenotype and functional properties, cells with clonal tumor-initiating capacity are called cancer stem cells (CSCs) or tumor-initiating cells (TICs) in [126]. The CSCs/TICs reside in specific niches in the tumor microenvironment that maintain their plasticity, protect them from immune defense mechanisms, and modify their metastatic potential [126]. In [127] MSCs was shown to interact with cancer stem cells (CSCs) or tumor-initiating cells (TICs), supporting several activities such as parenchymal cell growth and increased resistance to therapy; cancer cell dormancy and evasion from the immune system [128]. These were made possible either through paracrine secretion in [126, 129], or gap junction contact [127]. Alternatively, MSCs can affect epithelial cancer cell function by direct contact, causing increased expression of microRNAs, such as mir199a and stem cell-associated factors in the epithelial cells [130]. Mesenchymal stem cells secrete various growth-supporting cytokines, growth factors, and microRNAs that in some cases are stored inside extracellular vesicular particles (exosomes) as indicated in [131]. Exosomes are small (40–100 nm in diameter) membrane-bound organelles that function as part of an intercellular communication mechanism and characteristically include various types of molecules, including: matrix metalloproteases [132]; molecules that activate signal transduction [133], oncomiRs, bioactive lipids, and metabolites [134]. It was demonstrated in [132] that during tumorigenesis, exosome-bound factors modify the phenotype of the epithelial cancer cells or tumor stromal cells to support the aggressive phenotype and tumor progression.

Tumor stromal production exhibits similar qualities as normal wound repair such as new blood vessel formation, immune cell and fibroblast infiltration, and considerable remodeling of the extracellular matrix as indicated in [135]. Additionally, it was further stated that the recruitment of local normal host stromal cells, such as bone marrow mesenchymal stromal cells, endothelial cells, and adipocytes, help create a conspicuously heterogeneous composition and that these cells secrete an abundance of factors that help regulate tumor development [135]. Potential targets for tumor-associated stromal cell recruitment have been identified in [135] to include the following host tissue: bone marrow, connective tissue, adipose tissue, and blood vessels. Moreover, evidence suggests that tumor-associated stroma is a prerequisite for metastasis and tumor cell invasion. These are known to arise from at least six different origins: immune cells, macrophages, adipocytes, fibroblasts, pericytes, and bone marrow mesenchymal stromal cells [135]. These are cells originally meant to defend the host tisues.

During the early stages of tumor development and invasion, the basement membrane is degraded, and the activated or reactive stroma, containing fibroblasts, inflammatory infiltrates, and newly formed capillaries, comes into direct contact with the tumor cells [136]. With this development, the newly formed stroma (activated stroma) is lodged between tissue planes of little or no resistance, thus, disrupts the continuity of normal structures and cleaving paths for the invading tumor cells–intramural stromatogenesis for endophytic tumors, with the basement membrane matrix

modifying cytokine interactions between cancer cells and fibroblasts [136]. As indicated in [137], these cancer induced alterations in the stroma, will contribute to cancer invasion. In animal studies, it has been demonstrated in [27] that both wounding and activated stroma provides oncogenic signals to facilitate tumorigenesis. This is evidenced in [138] which reported that although normal stroma in most organs contains a minimal number of fibroblasts in association with physiologic ECM, the activated stroma is associated with more ECM-producing fibroblasts, enhanced vascularity, and increased ECM production. It is important to note that certain types of skin cancers (basal cell carcinomas) cannot spread throughout the body because the cancer cells require nearby stromal cells to continue their division, besides, the loss of these stromal growth factors when the cancer moves throughout the body prevents the cancer from invading other organs [138]. Stroma is made up of the non-malignant cells, but can provide an extracellular matrix on which tumor cells can grow and stromal cells may also limit T-cell proliferation via nitric oxide production, hindering immune capability [138].

V. CONCLUSION

This work succeeded in showcasing the importance of stroma in health, tumorigenesis and in cancer development and progression. Indispensability of the stroma in nurturing epithelial tissue by effective functions of the tissue stromal cells to maintain tissue homeostatic balance was highlited. Normally, the molecular interation between epithelial cells and other cells in stromal compactment are physiologically made to be intact. So any alteration as a result of cellular perturbation would disrupt the molecular interaction and thus obstruct the whole system. The intact of the stromal compartment, in fact is a strong hold for the whole body functional system, with stromal cells possessing anti-tumor effects. Despite all these homeostatic and defensive integrity of the stroma and the cells, over-whelming mutational load induce changes and thus promoting neoplasia and dysplassia and subsequently cancer and metastasis. This indicates that the original natural features of tissue stroma, is protumor only by expression of loss of function and gain of function to promote tumor/cancer. This work indicated that the tumor/cancer cells being an entity, desiring autonomous growth and entire developmental pathway, sees tissue stroma as a refuge, framework and highly patho-physiologically dependable for initiation, progression and metastasis and insists on its colonization as a fertile farmland to grow on. From all indications, cancer development cannot hold without colonizing or engaging the tissue stroma.

Evidences were shown that creation of tumor stroma is not part of human development but after effect of multiple or accumulated genetic and epigenetic mutations in epithelial cells, thus stroma overwhelmed by tumorigenesis (tumor microenvironment) emanate due to genetic changes that ushered in numerous neo-antigens or foreignness into the surrounding tissues thus inducing pathophysiological activities that could overwhelm the defensive function of the stromal system and shift the functions to aid tumorigenesis. This development could be redressed if the causal effects are resolved by the defensive and physiological/DNA repair arsenals of the body.

This work indicated that the stroma and the stromal cells are like farmland and the soil nutrients respectively. The accumulated alterations in cancer cells mediate functional changes on a framework or scaffold (the stroma) that serves for healthy physiological maintenance. This is the first beneficial thing for cancer development and/or progression, to initiate dependable bedrock and being more aggressive, hijack the multifunctional roles of the stromal cells (the nutrients) for its developmental process. Hence it is here indicated that tumor microenvironment is like a garden or orchard formed, carved out from a farmland (stroma) and uses the farmland nutrients to grow its crops. This is what I referred to as stromal colonization and stromatogenesis for cancer progression.

I uphold the existing 10 hallmarks of cancer by [1, 2] and I key in with their definition of hallmark, thus I maintain that stromal colonization and

stromatogenesis is an acquired functional capability from mutational changes or genetic alterations that induced abnormal and aggressive behavior on the transformed cells, aggressive enough to overwhelm and polarize the resisting or defensive functional nature of the stroma cells to suit its autonomous progressive tendencies. Hence the stromal and immune cells polarization, emanate phenotypic cells types with reversed cell functions. It is important to note that prior to the clearly established pathological, radiologic and diagnostic observations, persistent inflammation en route chronic inflammation existed, indicating a prolonged engagement of the stromal tissue by the minor genetic and epigenetic cells alteration en route multiple and accumulated genetic and epigenetic cell damages which induced chronic inflammation and colonization of the tissue stroma. The enomous role of persistent immune complexes resulting from endogenous danger molecules in mediating this chronic inflammation, have earlier been indicated in [139]. My understanding of cancer development is that the development is neither automatic nor acute but trails prolonged battles with mutational developments in the surrounding tissues. In context of this study therefore, tumor microenvironment is defined as an acquired developmental niche carved out from normal tissue stroma by severely altered tumor cells due to accumulated cell mutation and epimutational alterations and colonize the surrounding environment in other to survive and metastasize. So, it is suggested here that acquisition of a base like scaffold or framework such as stroma is an important developmental phenomenonal characteristic of cancer initiation and metastasis. Tumor microenvironment is to tumor as ranch is to cattle or garden to a plant. Many authors have enphasized on the utmost importance of the stroma dating as far back as 1889 by Paget, 1863, by Virchow, 1913, by Levin. It is important to honour these men posthumously by adopting tissue stroma colonization and stromatization as one of the trademarks of tumor/cancer.

List of abbreviations

ECM---- Extracellular matrix
MSCs---- Mesenchymal stem/stromal cells
TAFs ---- Tumor-associated fibroblasts
NME---- Native Microenvironment
RME---- Regenerative microenvironment
TME---- Tumor microenvironment
NOD-SCID---- Non - obese diabetic severe combined immunodeficient
ISCT- International Society for Cellular Therapy
CAFs---- Cancer associated fibroblasts
CRS---- Cytokine release syndrome
SPARC---- Secreted protein acidic rich in cysteine
PDGF---- Platelet derived growth factor
RMFs---- Regenerating myealinated fibres
HGF---- Hepatocyte growth factor
TGFβ---- Tumor growth factor beta
DCIS---- Ductal carcinoma *in situ*
MMPs---- Matrix metalloproteinases
PDGF-BB---- Platelet-derived growth factor BB
bCSC---- Breast cancer stem cells
ALDH---- Aldehyde Dehydrogenase
SOX2---- Sex determining region Y Box 2
OCT4---- Octamer-binding transcription factor4
NANOG ---- homebox protein Nanog
KLF4---- krupel like factor 4
EMT---- epithelial mesenchymal transition
TNBC---- Triple negative breast cancer
CCL2---- Chemokine ligand 2
FGF5---- Fibroblast growth factor 5
TICs---- Tumor-initiating cells

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