



Dissecting Biology of Solid Tumour: The Microenvironment and Cancer Progression

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Authors' contributions

This work was carried out in collaboration between both authors. Author MO designed the study, formatted the work, and wrote the first and second draft of the manuscript. Author OBO managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript

Review Article

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ABSTRACT

Focus on cancer therapy is experiencing a major paradigm shift from ways of attacking tumor cells to a strategy for specifically targeting the tumor microenvironment (TME). This approach requires a comprehensive understanding of roles of each component of the tumor environment. A description of the tumor microenvironment and its impact on tumor progression is presented here. Available studies indicate that both tumor/epithelial and stroma characteristics play important roles in cancer progression. Details of this work show that different components of the tumor microenvironment contribute towards cancer progression and clearly suggest a role for use of combination therapies for tight tumor control.

Keywords: *Cancer microenvironment; tumor progression; hypoxia; cancer resistance; metastasis and autophagy.*

1. INTRODUCTION

The stroma is the structural framework that provides support and enhances cell-cell interactions and normal tissue homeostasis [1]. The stroma is often separated from epithelium by the basement membrane, which is an amorphous dense sheet-like structure usually lying basolateral to the epithelium and endothelium [2]. The basement membrane separates tissues into compartments, and is always in close contact with cells [3]. The basement membrane has been shown to be similar to the ECM - which consists of the basement membrane and other components like fibronectin, collagens, heparin sulphate proteoglycans (HSPGs) and laminin. These elements link up to form a three dimensional matrix collectively known as the interstitial matrix that helps to prevent and restrain neoplastic transformation in a normal tissue [3]. Also found within the matrix is the matrix degrading enzymes such as proteases, matrix metalloproteinase (MMP) which in tumors are known to be necessary for angiogenesis and invasion. In this paper we review available studies that draw on understanding the role of tumor microenvironment on cancer progression. This review aims to dissect solid tumors to present a better understanding of the cellular and molecular markers in the tumor microenvironment that may represent potential drug targets for improving cancer treatment.

1.1 Fibroblasts

Another important member of the stroma is the fibroblast. A large body of evidence, including co-culture experiments and genetic studies has shown that stroma fibroblasts are involved in preventing tumor progression of transformed epithelial cells through the release of TGF β . However in contrast they promote tumor progression of initiated prostate epithelial cells [4,5]. [6] reported that there is a difference between normal tissue fibroblasts and resident tumor fibroblast. She reported that the tumor fibroblast when activated secrete highly proliferative markers including the fibroblast activation protein (FAP) otherwise called myofibroblasts that promotes malignant epithelial transformation. In fact, evidence from published studies suggests that the state of fibroblast in the microenvironment determines which aspect of its bipolar function would override the other [7]. However, the overall role of fibroblast in malignant progression and treatment failure is yet to be fully elucidated.

1.2 The Vasculature

It has been established that a tumor cannot growth beyond 150 – 200 μ m without formation of new blood vessels to deliver sufficient oxygen and nutrients to the dividing cells. The process by which the new blood vessels are formed is called angiogenesis. This involves the growth primarily of endothelial cells and the associated cell, the pericytes. The main factors controlling this process are vascular endothelial growth factor (VEGF) and the angiopoietin/Tie-2 system [8]. The new blood vessels are initially small, consisting initially of only the endothelial cells (Fig. 1). The increase in VEGF has been shown to be positively correlated with aggressive growth in breast cancer, prostate cancer and stomach cancer [9]. Previous studies have indentified other factors in the tumor microenvironment that possess angiogenic potential, these include interleukin 8 (IL-8), epidermal growth factor (EGF) and angiogenin [9,10,11]. Data from the author's laboratory provides a strong link between intra-tumor oxygen level and angiogenic responses [12]. Generally, intra-tumor oxygen play a major role in up regulation of pro-angiogenic factor such as VEGF-A – an endothelial cells' specific mitogen [13]. In fact, the transcriptional regulation of VEGF-A is mediated by hypoxia inducible factor 1 alpha (HIF-1 α) [14]. Hypoxia induced up regulation of VEGF – A

has been associated with increased expression of VEGFR, which in turn results in increased angiogenesis [14]. Data from clinical studies have shown sufficient correlation between angiogenesis and progressive tumor growth and metastasis. It appears that the link between angiogenic responses and tumor oxygenation in the tumor microenvironment may be associated with the nature of existing vasculature in the tumor which has a particular abnormal feature including decreased inter-endothelial cell junctions, chaotic blood flow, incomplete endothelia lining, and paucity of pericytes [[15,16]. Thus the oxygen delivery state of solid tumor is severely compromised by irregular oxygen diffusion geometry, structural abnormalities of blood vessels; this might perturb microcirculation in the tumor, culminating in heterogeneous distribution of oxygen within the tumor [14,16].

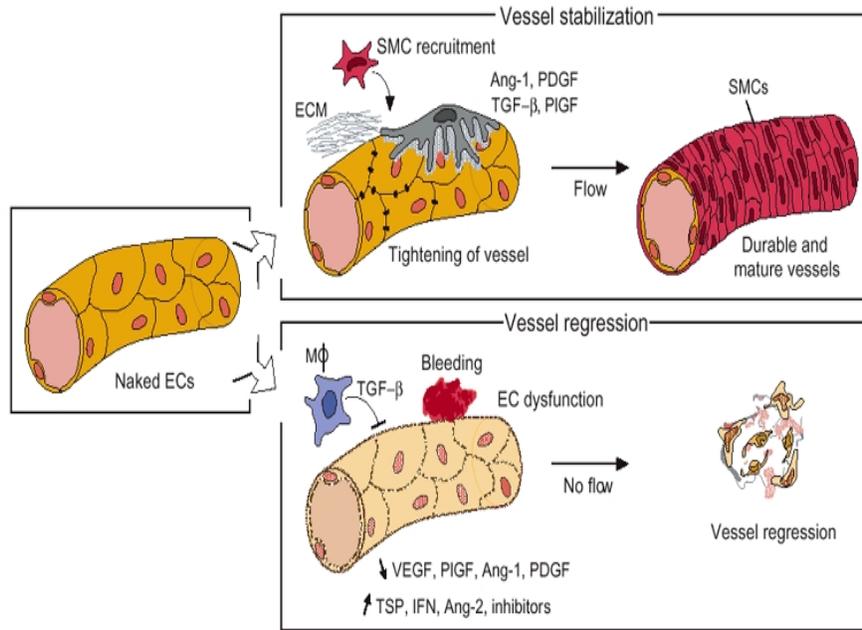


Fig. 1. Showing the processes involved in the formation, and regression of blood vessels. The initial step involves the formation of a tube-like structure by the endothelial cells (EC), followed by the tightening of the vessels through the actions of smooth muscles cells, extracellular matrix and growth factors. (with permission from [8])

1.3 Tumor Cells and Stroma

A solid tumor is made up of a complex cellular system consisting of stroma and tumor cells. Perhaps a well coordinated signalling between these compartments might have been affected by the tumor micro environmental milieu. In recent years the importance of the tumor microenvironment in malignant progression and treatment sensitivity has become more widely recognised. The tumor microenvironment consists of many components including stroma cells like fibroblasts, endothelial cells and pericytes which form the blood vessels, infiltrating cells such as macrophages, and extracellular matrix (ECM) components (e.g. laminin, collagen) [17,18]. In addition many small to medium sized molecules are produced/accumulate in the tumor milieu, and frequently they are distributed unequally

across the tumor. E.g. growth factors, oxygen, pH cytokine, chemokines [18]. Distinctive features of the tumor microenvironment are hypoxia, low extracellular pH and low glucose concentration. Recent studies show that the tumor microenvironment exerts a critical influence that could decide the fate and phenotype of solid tumors [19].

2. TUMOR MICROENVIRONMENT AND CANCER PROGRESSION

There is considerable evidence suggesting that the tumor microenvironment plays a critical role in cancer progression of cancer through a number of complex cellular interactions involving stroma cells [18, 19]. However, the exact mechanism by which this occurs is not clear. Recently, a micro dissection of tumor for global gene expression analysis identified 129 up-regulated and 21 down regulated genes within the tumor microenvironment mainly linked to remodelling of the ECM, infiltration of macrophages and neutrophils, and increased angiogenesis to promote malignant phenotypes in the endothelial and tumor cells. This study clearly showed that abrogation of the tumor suppressive function of PTEN in tumor stroma activates Ets 2 (Oncogenic transcription factor genes of the *ETS* family which include the four *ETS* family genes *ERG*, *ETV1*, *ETV4* and *ETV5ETS*) in both fibroblast and tumor cells with resultant transcriptional up regulation of MMP -9, which is known to mediate release and activation of matrix bound VEGF-A to VEGF₁₆₄. VEGF₁₆₄ has been widely recognised as a specific ligand for VEGF receptor 2 (VEGFR2; FLK-1; KDR) and the most potent effector of VEGF mediated endothelial cell activation and angiogenesis [20].

Also, this author showed a strong link between Ets activation in stroma, epithelial cell, MMP-9 activity in the ECM, and activation of tumor vasculature and suggests that stroma PTEN – Ets pathway operates to effect tumor suppression; hence, ETS-2 is a key regulator of tumor cell growth and angiogenesis. Furthermore, cross talk between these cells may also be initiated by the redox status of the micro-environment. There is evidence showing that hypoxic stress can induce multiple changes in gene expression and selection for cancer cells with aggressive genotypes and phenotype [21,22]. The actual mechanism of this process remains poorly understood though there are sufficient indications that this may operate at least in part through specific oxygen sensitive pathways including those controlled by hypoxia inducible factor 1 (HIF -1), mammalian target of rapamycin mTOR kinase axis and the unfolded protein response UPR pathway [23]; see chapter 1.xfor more detail). When activated, the downstream signalling in these three pathways can bring about altered gene expression, loss of apoptosis, and increased angiogenesis and tumor progression.

2.1 Adhesion Molecules

The ECM has been described as an insoluble network of proteins that are secreted, assembled and remodelled by cells. The ECM may exert its effects in the microenvironment in part through the integrins, Fig. 2. Integrins are made up of heterodimeric receptors that link the resident cells to the components of ECM and are often involved in intracellular signal transduction [1]. These protein complexes are expressed by tumor cells and include $\alpha\beta3$, $\alpha\beta5$, $\alpha5\beta1$, and $\alpha6\beta4$. Some clinical studies have highlighted the complex role of integrins in cell switching to a cancerous phenotype [24,25]. Although the contribution of oncogenes to this phenomenon has been mentioned above, aberrant expression of integrins has been shown to be linked to cancer progression, Fig. 2. Studies suggest that neoplastic cells during transformation lose their integrins that secure them to the basement membrane [26]. Conversely transformed cells often over express certain integrins at the later stages of their

development; this is thought to promote their survival and migratory potential during invasion and metastasis [25,26].

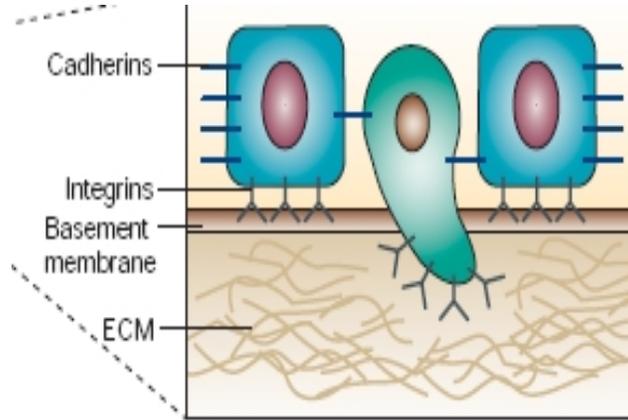


Fig. 2. Schematic diagram showing the molecular and cellular (integrin and Cadherin) changes in tumor cell environment at the onset of cancer invasion. The central transformed cell shows loss of cadherin linkages to the neighboring normal cells and abnormal integrin binding to the basement membrane (with permission from [3]). This is one of the early events that might occur to allow cancer cells undertake epithelial to mesenchymal transformation; this is a hallmark of metastatic process in a given tumor. The morphological changes in the figure are obvious in the 2 cell in the middle compared to the cell in the right and left

In addition, the joint action of integrins and tyrosine kinase receptor (RTKS) signalling pathways, which promote tyrosine phosphorylation components of E-cadherin and beta catenin, have been associated with loss of cell – cell adhesions in cancer cells and tumor progression. Loss of E-cadherin, a cell–cell adhesion molecule, has been reported in several invasive and metastatic cancers (Fig. 2). For example, one of the earliest reports of the role of E-cadherin in tumor invasion was by [27]. In this study, a cDNA encoding E-cadherin was transfected into highly invasive tumor cell lines. It was observed that these cell lines while showing high expression of E-cadherin lost their invasive activities which were recovered by treatment with anti E-cadherin antibody. Subsequently, further studies confirmed that loss of E-cadherin mediated disruption of cell-cell adhesion (Fig. 3) which in part promotes malignant tumor progression and mediates epithelial mesenchymal transformation (EMT) [28].

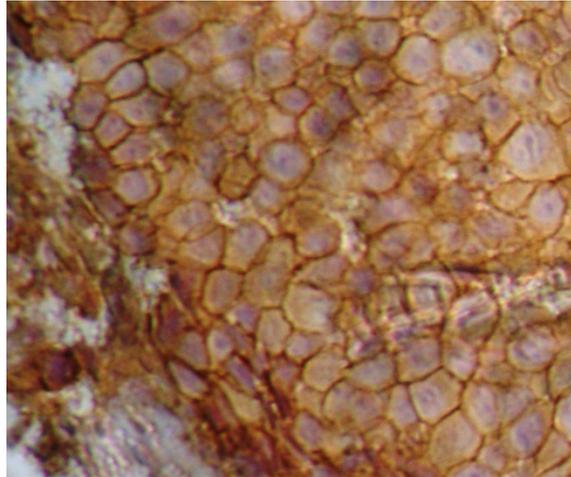


Fig. 3. Showing cell-cell connections with immunohistochemical expression of E-cadherin molecules which are predominantly at the membrane of prostate epithelial cells in a tumor xenograft. Produced by Dr Maxwell Omabe

EMT is a process whereby epithelial cells lose their polarity and cell – cell junctions and acquire migratory characteristics [29]. Though this process is well recognised in normal early development in which it operate to enhance cell dissemination in vertebrate embryos [30], however, studies demonstrated that epithelial cells close to the site of injury changed their phenotype and disassociated from the epithelial cells from which they had originated and migrated to close the injury [31]. Recent studies now show that tumor cells exploit the mechanism of EMT to disseminate to distant organs [32,33]. Usually, cells in a normal tissue are physically and functionally connected in such a way that the integrity of the cell junctions are preserved (Fig. 3), however, studies suggest that changes in tumor tissue phenotype occur through the process of EMT resulting in cells reorganizing their polarities, actin cytoskeleton to generate traction and protrusion force for motility [32,33]. Typically, the cell lose expression of E-cadherin to abrogate cell – cell junction (Fig. 3) and adopt a mesenchymal – like phenotype to favour detachment, initiation of single cell movement, invasion, motility and metastasis [33].

Usually, epithelial cells in tissues maintain a robust cell-cell adhesions; this is mediated by cytoplasmic cadherin domain often bound to the β catenin and linked up with the actin cytoskeleton by the catenin. Classic cadherins include E-cadherin, N – cadherin, and P – cadherin; these are transmembrane proteins usually expressed by the basal epithelial cells [33]. While E-cadherin is strongly expressed by these cells, P –cadherin often shows weak expression. Evidence strongly suggests that reduced expression of E-cadherin is associated with high cancer grade, advanced stage and poor treatment outcome profile [33].

2.2 Growth Factors

Not surprisingly a large number of growth factors are known to play a role in the cross talk between cells and ECM components in the tumor microenvironment. Transforming Growth Factor beta (TGF- β) is one of the growth factors found in the tumor microenvironment. It has been reported to have promalignant and anti-malignant activities. TGF- β is a potential inhibitor of epithelial cell growth and mammary gland cell proliferation. Its inhibitory action is

induced through type I and type II TGF- β receptors, and the Smad mediated and non-Smad mediated cell signalling. However, a mutation in either TGF- β receptors or Smad signalling pathways or both may be responsible for its promalignant function. Moreover, TGF- β has been shown to mediate cell transition from epithelia to mesenchymal cells and promote angiogenesis [26,33].

Tumor necrotic factor alpha (TNF- α) has been reported as another important factor in the tumor microenvironment and was earlier known to induce apoptosis in tumor cells [34]. However, recent studies showed that TNF- α expression was associated with tumor progression, and that its expression correlated with tumor prognosis in prostate cancer [35,36]. TNF- α , usually secreted by macrophages stimulates the production of monocyte chemo attractant protein I (MCP-I- CCL2) by resident tumor cells in the microenvironment [37], which in turn induce infiltration of more macrophages into the microenvironment; causing a form of vicious circle effects. More biological functions of TNF- α have been reviewed by [35,37], and included induction of interleukin 1 secretion and promotion of angiogenesis. However, there are some conflicting reports concerning its functions, since it was found to exert pro-apoptotic and proliferative effects in different forms of cancer.

3. VASCULAR CHANGES AND CANCER STEM CELLS

There are evidences indicating that some cancer cells possess stem cell characteristics [38]. In fact, the contributions of cancer stem cells (CSC) in the growth and development of various tumors have been widely recognized. Recently, [39] compared pattern of cell division between CSC and normal stem cells; and showed that both symmetrical and asymmetrical cell divisions occurred in CSC while asymmetrical cell division occurred only in normal stem cells - asymmetrical cell division would result in production of progenitor cells while symmetrical cell division would give rise to two daughter cells responsible for populating the tumor. The study shows that tumor volume may be populated by CSC which possesses a decreased P53 activity by shifting the usual asymmetric cell division to symmetric type which has a consequent doubling of the cell number during cancer growth and development. [40] has shown that the CSC accumulates mostly at the tumor edge and remains immobile initially until further transformation or remodeling by takes place.

The role of angiogenesis and cancer progression has been widely reported in all types of cancer including endocrine and neurological cancers. Evidence has shown that increased angiogenesis commonly measured clinically as microvascular density, may serve as an indication for aggressive tumor progression and poor response to therapy [41]. In the management of endocrine cancers such as breast and prostate cancer, hormonal ablation is a well recognised strategy for tumor control [42,43]. This treatment has been shown to result in tumor regression, delay of disease progression and overall benefits in quality of life in patents shortly after the therapy. Mechanistic studies have shown that this treatment interferes with tumor vasculature. In fact both animal and clinical studies have demonstrated that hormonal ablation therapy results in vascular collapse, apoptosis and tumor regression [42,44]. Evidence suggests that the vascular collapse occurs as early as 24 hours after treatment. This phenomenon is commonly followed by another episode of vascular re-growth and aggressive tumor progression [41,44]. In fact, this second wave of tumor growth is often characterized with global changes in the tumor microenvironment such as hypoxia, genomic instability, and increased in tumor cell's invasiveness and increased in angiogenesis. It is not known what molecular changes are in particular responsible for the second episode of tumor growth. Emerging evidence indicates that hypoxia may exert a selective pressure which may result in some cell clones with altered genetic signature which allowed them to resist

treatment in these tumors [44]. While this may be one of the contributing factors, it is not known why endothelial cell proliferation is particularly affected at this time point.

3.1 Stem Cells and Tumor Progression

It is clear that cancer stem cells play important role during cancer progression. In fact there are sufficient evidence demonstrating the presence of cancer stem cells and their contributions in prostate and breast cancer progression. Emerging evidence from recent studies has now shown that cancer stem –like cells can differentiate into endothelial cells and thus contribute into formation of new vasculature [45,46]. These authors independently showed that glioblastoma cells and endothelial cells contained expressing their specific markers had similar genomic alteration suggesting that they were of the same neoplastic origin. In a glioblastoma tumor, [45,46] used stem cell specific marker CD133⁺ and selected sub cell clones which were positive for the marker. The author further demonstrated that the CD133⁺ cells contained a subset expressing endothelial cell markers CD144 known as vascular endothelial cadherin which poses characteristics of endothelial progenitors. Evidence from linear analysis and clonal studies also demonstrated that the CD133 cell were multipotent and differentiate into both tumor cells and endothelial cell types. It is widely recognised that endothelial cells growth and development is dependent on the availability of vascular endothelial growth factor. In this regard, whenever, the cells were blocked of availability of VEGF, the differentiation of CD133 cells to endothelial cell types was not affected; but when the CD133⁺ CD144⁺ cells were injected into a nude mice, an aggressive tumor developed which showed maximum level of tumor vascularisation s compare to the tumor that developed after CD133⁺ CD144 – cells were transplanted confirming that the increased in angiogenesis may be induced by the cells expressing the endothelial cells progenitor. These papers clearly show novel mechanisms for tumor angiogenesis and cancer progression, and suggest that stem cell like cells in tumor may be a potential target in progressive and invasive cancer.

4. TUMOR MICROENVIRONMENT, WARBURG EFFECT AND AUTOPHAGY

Autophagy or self-digestion of cells is activated upon various stressful stimuli and has been found to be a survival and drug resistance pathway in cancer. However, genetic studies support that autophagy can act as a tumor suppressor. Furthermore, defective autophagy is implicated in tumorigenesis, as well [47].

In the early 1920s, Otto Warburg, a Nobel Laureate, formulated a hypothesis to explain the "fundamental basis" of cancer, based on his observations that tumors displayed a metabolic shift toward glycolysis [48]. In 1963, Christian de Duve, another Nobel Laureate, first coined the phrase auto-phagy. First, cancer cells are known to operate in a particular state of hypoxia; this often results in production of increased level of hydrogen peroxide [48]. Then, as a consequence, oxidative stress in cancer-associated fibroblasts drives autophagy, mitophagy, and aerobic glycolysis. In fact, recently published evidence clearly showed the metabolic relationship between cancer cells and the fibroblast in driving tumor progression [49]. Clearly, the authors proposed a new paradigm to explain the compartment-specific role of autophagy in tumor metabolism. In that model, autophagy and mitochondrial dysfunction in the tumor stroma was shown to induce autophagy and promote cellular catabolism; this results in production of recycled nutrients. These chemical building blocks and high-energy "fuels" would then drive the anabolic growth of tumors, via autophagy resistance and oxidative mitochondrial metabolism in cancer cells. Clearly this new form of stroma-epithelial

metabolic coupling: "two-compartment tumor metabolism model [49] may explain why cancer cells resist chemotherapy and apoptosis. In a mechanistic study, [49] genetically created autophagic fibroblasts which were generated by allowing the cells to stably over express key target genes that lead to AMP-kinase activation, such as damage-regulated autophagy modulator (DRAM) and liver kinase B1 (LKB1; critical regulator of cellular stress and the autophagic response [49]. In addition, autophagy-resistant cancer cells were also derived by over expressing GOLPH3, which functionally promotes mitochondrial biogenesis. It is known that GOLPH3 is a mitochondrial protein which critically regulates mitochondrial lipid biogenesis by shuttling between the Golgi apparatus culminating in an increase in the delivery of mitochondrial phospho-lipids (such as cardiolipin) which would result in overall increase in mitochondrial mass [49]. The authors demonstrated that DRAM and LKB1 over expressing fibroblasts were constitutively autophagic and effectively promoted tumor growth. The experiments provided data which strongly support Warburg effect, suggesting that autophagic fibroblasts showed mitochondrial dysfunction, with increased production of mitochondrial fuels (L-lactate and ketone body accumulation). However, in the same study, GOLPH3 over expressing breast cancer cells were autophagy-resistant, and exhibited signs of increased mitochondrial biogenesis and function, which resulted in increased tumor growth. Thus, autophagy in the tumor stroma and oxidative mitochondrial metabolism (OXPHOS) in cancer cells may both dramatically promote tumor growth, independent of tumor angiogenesis [49]. The precise impact of autophagy on malignant transformation has not yet been clarified, but recent data suggest that this complex process is mainly directed by metabolisms in the different cell types in the tumor microenvironment. Conclusively, relationship between autophagy and cancer progression under the influence of the tumor microenvironment may indicate a novel aspect in cancer chemotherapy [47,49].

5. CONCLUSION

In conclusion, emerging evidence from microRNA expression profiles studies [50] confirms that changes in the tumor microenvironment including decreased miR-31 can reprogram normal fibroblasts into tumor-promoting cancer-associated fibroblasts. Detail of this has been discussed above. It is now clear that perturbation in the stroma microenvironment can affect tumor growth. This represents an important therapeutic target.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

SUBMISSION DECLARATION

The authors declare that no part of this work has been published elsewhere or is awaiting publication anywhere.

COMPETING INTERESTS

I declare no conflict of interest, and that no fund from any funding body was made available for this work. This study was funded by the author only.

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