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The tumor microenvironment as a target for anticancer treatment

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Abstract:

Cancer is generally considered as a (pathological) microcosm emerging from a deregulated genome occurring in a single cell. Yet, this paradigm has encompassed several shortfalls, and it is now increasingly recognized that the overall framework is very much complex as previously thought. Indeed, the cell-stroma interaction plays a pivotal role in both cancer onset and prevention, by providing a set of biophysical and biochemical cues, which drive cell towards neoplastic transformation, eventually leading to metastasis. However, experimental and clinical evidences suggest that cancers can be induced to become quiescent, differentiate, die or form completely normal tissues, if provided with the correct set of complex signals, as conveyed by embryonic tissues or other microenvironmental cues. These data suggest that by manipulating cancer microenvironment may help in opening new avenues for therapeutic solutions.

Key words:

Cell-stroma interactions, tissue organization theory of carcinogenesis, tumor microenvironment

Introduction

It is generally accepted that cancer is an irreversible disease that results from accumulated gene mutations and chromosomal abnormalities. This model has been proposed by the somatic mutation theory of cancer (SMT), which envisions understanding cancer at the cellular level of organization, while claiming that cancer is a problem of regulatory control of cell proliferation and invasiveness, mainly due to mutations and/or deregulation of special classes of genes, that is, oncogenes and suppressor genes.^[1] If cancers are caused by irreversible genetic changes, even accumulated during the process which leads from the initiation to the metastatic spreading of cancer, treatment will require that these cells must be killed or surgically removed to achieve a successful cure.

However, current treatments, grounded on the aforementioned SMT paradigm are disappointing and demonstrate that no further improvement can be obtained by conventional chemotherapy, and only slight advantage in overall survival rate has been reached since the introduction of the so-called target (gene)-based drugs.^[2] Indeed, despite some progress, conventional as well as “innovative” chemotherapeutic protocols failed in obtaining significant improvement in overall survival, given that the death rates from the prevailing form of tumors (breast, colon, and prostate cancers) show, at the best, a stable trend.^[3] In a broader sense, there is a perception that cancer research is not progressing as fast as might be hoped from the investment in

research.^[4] This evidence prompted someone to suggest that we are losing the “war on cancer”.^[5]

Even SMT supporters have recently acknowledged that the gene-based paradigm is facing increasing shortfalls and contradictions. Accordingly to Weinberg, the expected evidence did not turn out to vindicate explanations provided by SMT. Quoting him, “half a century of cancer research had generated an enormous body of observations [...] but there were essentially *no insights into how the disease begins and progresses*”.^[6] Despite the expectations raised by “the Ames’ axiom (“substances act as carcinogens because they have mutagenic activity”), it shortly turned out that most powerful carcinogens are actually not mutagen”; “but fortunately-as Weinberg candidly admits – *I and others were not derailed by discrepant facts*”. Indeed, a whole series of “discrepant facts” were ignored, while acknowledging that their realistic evaluation would have flawed the dominant paradigm.^[7] Yet, despite this realization, the search for mutated oncogenes and/or tumor suppressor genes continued unabated up to the present. “However even this was an *illusion*, as only became apparent years later the identities of mutant cancer-causing genes varied dramatically from one type of tumor to the next. Each tumor seemed to represent a unique experiment of nature”.^[6] Moreover, the ever-changing genomic portrait, the high genomic instability and heterogeneity of cancer, represent an insurmountable hurdle,^[8] and makes almost impossible to achieve a successful personalized, gene-based treatment.^[9] Indeed, experimental data provided so far urge us to revisit the role of gene mutations in cancer,

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Submission: 08-10-2014

Accepted: 17-10-2014

“suggesting that mechanisms for cancer initiation are broader than is typically thought.”^[10]

The tumor microenvironment

A plausible, testable, alternative theory of carcinogenesis has been proposed already 15 years ago – the Tissue Organization Theory of Carcinogenesis (TOFT) – claiming that cancer arises from the deregulated interplay among cells and their microenvironment.^[11]

Epithelial cells can become dysfunctional if their microenvironment is severely perturbed. This control of epithelia by their stroma is a default condition in normal tissues, as adult organs do not change the composition, size or shape by uncontrolled remodeling. Therefore, rather than being a passive reaction to the cancer cell, the microenvironment might be a primary active factor in determining whether dysfunctional epithelial cells will be subject to neoplastic transformation, invading and further disrupting their local *milieu*, or, alternatively, merely become an indolent micro-hyperplasia or even be eliminated.

According to TOFT, the microenvironment represents the physical-biochemical support of the morphogenetic field, which drives epithelial cells toward differentiation and phenotype transformation, according to rules understandable only by means of a systems approach.^[12] Not only the microenvironment-cells interplay is a matter of “signaling interaction”, but also, it involves biophysical factors and field-based effects, usually, overlooked by the current scientific mainstream.^[13] Lesser attention has been given to the explicitly highlighted premise of TOFT that states that the default state of all cells is *proliferation*. Notwithstanding, by challenging the implicit premise included in SMT that assumes that the default state of cells in metazoans is *quiescence*, it makes TOFT stand-alone from other theories of carcinogenesis. Briefly, TOFT considers that the cancer is a tissue-based disease and adopts proliferation as the default state of all cells. This latter aspect of TOFT has been discussed at length elsewhere.^[14]

Studies on tumor microenvironment are dating back even from 1940, when microenvironment was shown to suppress skin carcinogenesis induced by chemical carcinogens.^[15] Since then, an increasing number of experimental data have demonstrated that the microenvironment actively contributes to carcinogenesis already since the first stages, by profoundly influencing many cellular processes such as growth, differentiation, and apoptosis.^[16,17] Discrete differences in extracellular matrix (ECM) composition and stiffness, selectively foster or inhibits proliferation, by modulating regulatory molecules of the cell cycle and the early response genes.^[18-20] The microenvironment regulates the transcription of genes associated with differentiating pathways^[21,22] and participates in shaping cells phenotypes, by modulating cell-stroma interactions and cytoskeleton architecture.^[23,24] Moreover, cell shape and microenvironmental cues may trigger programmed cell death signals, hence driving cells towards apoptosis.^[25,26] Changes in the microenvironment three-dimensional network or composition may lead to tissue fibrosis, augmented collagen cross-linking, and tissue stiffening, which have been associated to an increased risk of developing cancer.^[27,28] It is not trivial to recall that aging is associated to increase in both tissue stiffness and cancer incidence.^[29] In turn, tissue fibrosis and modification

of physical-chemical properties of ECM may likely influence tumor onset and progression by regulating soluble factors involved in inflammation^[30] and angiogenesis.^[31]

Participation of microenvironment in carcinogenesis is further highlighted by the observed changes in the phenotype of stromal cells residing within the tumor microenvironment. Both cancer-associated fibroblasts^[32] and endothelial cells,^[33] have been demonstrated to carry structural changes^[34] and molecular abnormalities that may actually foster cancer onset and development.^[35-37] Exposition of stroma to the chemical carcinogen induces stroma-dependent changes in epithelial cells towards cancerous transformation, by disrupting the normal stromal-epithelial interactions, whereas by treating directly the epithelial cells does not lead to any neoplastic transformation.^[38] Conversely, the malignant phenotype of tumor cells may be efficiently counteracted, eventually leading to a fully neoplastic reversion, by placing cancer cells into a normal three-dimensional-microenvironment, which provides appropriate biochemical and biophysical stimuli.^[39,40] Similar results have been confirmed by many other studies,^[41,42] thereby outlining that the main factor in driving the carcinogenic process lies on the cross-talk in between microenvironment and epithelial cells.^[43]

Tumor Microenvironment as a Target

Such results are highly exciting and intriguing, as they suggest that, contrary to what has been thought until now, cancer can be actually viewed as a reversible process.^[44] Moreover, conversely, if cancer is a reversible process, then the entire carcinogenic paradigm must be changed accordingly.^[45] This is a highly provocative idea, but perhaps is the only opportunity to deal with cancer complexity and find an effective treatment.

Indeed, an increasing and compelling body of evidence – provided by experimental studies and corroborated from clinical studies – suggests that cancers can be induced to become quiescent, differentiate, die or form completely normal tissues, if provided with the correct set of complex signals, as conveyed by embryonic tissues or other microenvironmental cues.^[46-48]

Moreover, many clinical reports show that different cancers can undergo a spontaneous regression, reverting toward a normal phenotype. Spontaneous disappearance or differentiation of animal and human cancers, such as stage-IV neuroblastoma, liver neoplasia,^[49] breast tumors,^[50] and many others, have been hitherto reported.^[51] Similarly, several studies have shown that by placing cancerous cells into a “normal” microenvironment – that is, by restoring a normal, strong morphogenetic field – the tumor phenotype may be reverted into a normal one, eventually promoting a *de novo* cell and tissue differentiating process. Cancer cells exposed to embryonic morphogenetic fields^[52-55] or cultured in three-dimensional-reconstructed biological microenvironment mimicking the normal tissue architecture, undergo entrenched processes of apoptosis and differentiation, eventually ending up into the reprogramming of a “normal” phenotype.^[56,57] Usually, this evidence is either “neutralized”, arguing that microenvironment may activate other unknown genetic pathways through unspecified ways or else ignored, by

considering it a “funny” exception. The truth is that within the theoretical boundaries provided by SMT such results cannot be explained, and actually its advocates do not even try to do so. Indeed, if cancer onset were truly an event due to the accumulation of mutations in a few key-genes, once the threshold has been crossed there would be no way back towards normality. On the contrary, such paradoxical data constitute a pivotal element of TOFT, which posits that the microenvironment represents the physical-biochemical support of the morphogenetic field which drives epithelial cells towards differentiation and phenotype transformation.^[58,59]

Cancer-reversion induced by the microenvironment has been frequently ascribed to some key-molecular morphogens or gene modification affecting cell components of stroma.^[60] Consequently a wide subset of specific drug has been designed to target selective components, including chemo-attractants produced by tumor-associated fibroblasts, angiogenic factors (Fibrinogen- β , vascular endothelial growth factor [VEGF]),^[61,62] fibrotic processes,^[63] metalloproteinases (MMP),^[64] urokinase plasminogen activator,^[65] integrins,^[66] focal adhesion kinases,^[67] TGF- β ^[68] and many others. Until date, some promising results have been obtained, even if such new attempts have been flawed by an approach that still relies on a reductionist-based framework.

Indeed, studies performed by engineering the tumor microenvironment and/or by treating the stroma with factors capable of rewiring its physical-chemical properties show that cancer formation can be promoted or reversed by altering the mechanics or structure of the ECM,^[24,69,70] by modifying the connective structure of tissue stroma^[38,71] or even changing the bioelectrical *milieu*^[72,73] or the systems physical properties by acting on the gravitational field.^[74] Engineered bio-mimetic scaffolds mimicking the embryonic ability in reverting cancer into normal epithelium, could allow obtaining materials to be applied at the tumor resection sites in order to prevent local tumor recurrences.

Thinking about the cancer microenvironment as a “systems target”, entails taking into consideration some key issue in order to achieve a biological and clinical result. Thus, we have to look closely to some meaningful example of cancer management and reversion induced by manipulating the tumor microenvironment in order to gain fruitful insights about the mechanism behind that process.

Tumor Reversion and Management

Microenvironment drives hematological malignancies

Some recent papers outline how, even without any mutations, changes in the hematological niche as well as microenvironmental defects in retinol-binding receptors, may efficiently induce hematological malignancies. These studies demonstrated that the microenvironment, and specifically non-hematological cells, plays a pivotal role in enabling bone marrow cells to undergo a proper differentiating pathway, eventually inhibiting the occurrence of myeloproliferative disorders (MPD). Indeed bone forming osteoblasts have been recognized as a critical component of the hematopoietic stem cell (HSC) niche.^[75] The bone marrow microenvironment contributes in regulating HSC differentiation and also in

preventing cancerous transformation.^[76] Indeed, an abnormal β -catenin distribution, leading to increased activated β -catenin levels in the nucleus of the osteoblasts, has been demonstrated to trigger changes in HSCs, enhancing a shift toward the cancerous phenotype, that is, inducing acute myeloid leukemia (AML). As β -catenin interacts with E-cadherin in transducing mechanic stimuli from the microenvironment to the cytoskeleton, one could hypothesize that abnormalities in β -catenin distribution will likely affect the overall cell architecture and the correlated functions. A tight link in between shape and biological function has been indeed observed by many studies. Those experimental results have been confirmed by investigations carried out on AML patients, showing nuclear (activated) β -catenin in osteoblasts and upregulation of Notch signaling in hematopoietic cells, whereas nuclear staining of β -catenin was absent in healthy controls.^[77] These findings showed that the modification in osteoblasts may influence the HSC niche and are sufficient enough to drive the development of leukemia in mice. Deregulated expression as well as abnormal localization of β -catenin within the nucleus has been observed in different cancers and currently that feature is thought as key factor in carcinogenesis given that β -catenin modulation could influence many systemic properties of both cancer cells and their microenvironment. Thus, down-regulating β -catenin and its downstream correlated pathways, may represent a new, promising target in cancer drug discovery. However, to our best knowledge, no attempts have been so far performed to identify a pharmacological “bullet” able not only in reducing β -catenin levels, but also in inhibiting its nuclear localization and further activation. Fortunately, a few selected natural compounds, belonging to the class of polyphenols (epigallo-catechins, gallic acid, quercetin, procyanidins and many others), usually extracted from green tea and grape seed, display such outstanding property: β -catenin in cancer cells treated with grape seed or green tea extracts undergo a cytoplasmic and cortical redistribution, while control cells exhibited the protein mainly in the nucleus. Moreover, after 48-h treatment the overall β -catenin expression was dramatically reduced; consequently, many other β -catenin-linked pathways were down-regulated.^[78]

Retinol and hematological malignancies

Retinol acid receptors (RAR) display a wide range of effects on both cancer cells. This effect is triggered by the Vitamin A derivative, all-trans retinoic acid (ATRA) the naturally occurring ligand for all subtypes of RARs. More intriguingly, however, lack of RARs within the hematological niche, induces and sustains the development of MPD. Indeed, the hematological malignancies triggered in RAR-null mice are not intrinsic to the hematopoietic compartment, but it is induced by the RAR-null microenvironment. In RAR-null mice, the architecture of the hematological niche, that is, the three-dimensional structure of the microenvironment, is profoundly disrupted: Histological sections of under calcified tibiae revealed significantly reduced trabecular bone and increased osteoclasts number. Release of some inflammatory cytokines – like tumor necrosis factor (TNF) – was concomitantly increased. As the myelodysplastic syndrome in such cases is partially inhibited by blocking TNF release, it can be argued that RARs deficiency promotes MPD also by fostering an inflammatory-like *milieu*. By analogy, even Vitamin A deficiency can result in myeloproliferation.^[79] Retinoic acid

receptors regulate many different gene products, and aberrant expression or function of RARs has been identified in many different diseased states. It is likely that there are multiple contributing factors to the microenvironment-induced MPS observed in RARs null-animals, but it must be stressed that in such model micro environmental deregulation can be deemed the “the sole causative mechanism for MPS”. In turn, it is of outmost interest that pleiotropic activities exerted by retinol derivatives have been demonstrated to efficiently counteract MPD and even some forms of leukemia, as the promyelocytic leukemia.^[80]

Embryonic microenvironment

Compelling evidence has demonstrated that embryonic fields as well as soluble morphogens extracted from embryonic, pluripotent cells or from stem cells may efficiently inhibit cancer growth, promote apoptosis or induce phenotypic tumor reversion, documented by morphological, biochemical, behavioral as well as metabolomic features.^[71] These effects have been observed *in vitro*^[55,81-84] and also confirmed in a few selected studies, mainly carried out on advanced cancers^[85] or on hepatocellular carcinoma (HPC) patients.^[86,87] Namely, a randomized study has ascertained a significant advantage in advanced HPC patients (refractory to previous multi-drugs chemotherapy), treated with proteins extracted from *Zebrafish* embryos. An objective response rate of about 67% was recorded in the treated arm when compared to the control group, leading to a long-lasting disease-free interval up to 20 months.^[88] However, it is quite puzzling to understand why those patients achieved a so remarkable response. Indeed, no single active component has been so far identified within the crude protein extract from *Zebrafish* embryo, or from other conditioned embryo *milieu* that could explain the observed pharmacological effect, even if antiproliferative as well as pro-apoptotic activities have been ascribed to the protein extract considered as a whole.^[89] Embryo and stem cell-dependent microenvironment may likely act by modifying some biophysical cues, as well as through soluble morphogens, involving several key pathways.^[90-92] Both of these mechanism act by reshaping the morphogenetic field in which cancer cells are embedded.^[93]

Collagen as a target

The overall architecture of the microenvironment, and specifically the three-dimensional structure of the ECM, is largely dependent to collagen concentration, posttranslational modification (leading to structural remodeling through cross-linking) and proteolysis. At all of these levels, collagen has been shown to be deregulated in cancerous tissues, resulting in an abnormal ECM architecture. These changes are likely to affect ECM stiffness, as well as many other biophysical properties (viscosity, pH, hydration), which in turn may profoundly affect molecules (hormones, nutrients) transport, cell adhesion to the substrate and cell cytoskeleton configuration as well. Collagen and ECM cannot be longer considered as an inert scaffolding protein and migration barrier, given their interaction with epithelial cells may efficiently modulate gene expression, enzymatic activity and eventually cancer transformation.^[94]

Analysis of human tumors has revealed indeed an association between collagen expression or collagen modifying enzymes

and poor prognosis,^[95,96] supporting the notion that collagen remodeling is highly relevant to human cancer progression. In turn, a wide range of collagen-targeting molecules and drugs have been hitherto demonstrated to significantly influence cancer onset and development. Ascorbic acid, inhibitors of hyaluronidase^[97] and of Lysyl-oxidase,^[98] as well as many polyphenols extracted from green tea or grapes,^[99] have been shown to modulate ECM, matrix composition and stiffness. Additionally, several experimental and clinical studies demonstrated that those compounds exert a significant clinical activity, both as chemopreventive agents as well as anti-cancer drugs.^[100,101] Namely, it is worth of noting that ascorbic acid, whose anticancer effects are usually ascribed to its anti-oxidant properties, exerts even a more relevant effect by ensuring proper collagen metabolism, matrix remodeling, MMPs inhibition, preserving by this way the “stroma homeostasis” through different, entrenched mechanisms.^[102-104] Moreover, even if some disappointing results have been reported, there is little doubt that Vitamin C displays an important chemopreventive activity^[105] and can lead to a significant clinical benefit in integrative oncologic settings.^[106,107]

Collagen structure may significantly influence several parameters and functions by modulating both matrix and cells stiffness. Indeed, a more entrenched network performed by the interaction among types I and IV collagen foster integrin-mediated cell adhesion to substrate and enhances tissue stiffness.^[108,109] Enhanced stiffness promotes cytoskeleton remodeling, and interstitial fluid pressure (IFP) increase, thus hampering drug and molecular flux.^[110] On the contrary, by decreasing collagen fibril size or matrix density through collagenases addiction, interstitial pressure drops and drugs delivery is raised.^[111,112] Consequently, improved drug responses have been achieved when the collagen content in tumors has been reduced.^[113]

Indeed, strategies to overcome the environmental barriers constituted by abnormal microenvironmental vascular structure or increased stiffness and viscosity, have yielded some promising results.^[114] The two phenomena are closely linked: Vessel normalization, ameliorate tissue stiffness and reduces the interstitial pressure and this result not only enhances the drugs uptake, but will likely normalize the micro environmental metabolomic fingerprint. The administration of VEGF-receptor2-blocking antibodies, platelet derived growth factor antagonists, and TGF β inhibitors, among others, effectively reduces IFP, resulting in enhanced drug uptake.^[115-117]

A renewed interest in collagen study in cancer is taking off, as abnormal expression, proteolysis and structure of the “collagen network” influence cellular functions to elicit multiple effects on tumors, including proliferation, initiation, invasion, metastasis, and therapy response.^[118]

However, how collagen changes precisely influences tumor onset and development is still challenging. Namely, there is an urgent need to correlate collagen modification to cytoskeleton dynamics and cell function, in order to develop tractable methods to manipulate biochemical composition, architectural features and mechanical properties of collagen. Given that some apparently contradictory results have been reported,

demonstrating that both increased^[119] and decreased^[120] deposition of collagen can be associated with cancer growth, it is mandatory to outline that the main effect of collagen are mediated by its network architecture and/or by the dynamics of its remodeling rather than solely by protein level. Consequently, engineers and cancer biologists are beginning to find together the way to reproduce synthetic work Scaffolds that can induce cultured tumor cells to better mimic the *in vivo* phenotype.^[121] These studies highlight how important is regenerating a 'stem cell niche' with correct physical and chemical properties, given that providing cytokines alone is far from be sufficient.^[122]

Chronic inflammatory processes and the microenvironment

The highly ordered sequence of events that undergo during inflammation and repair may become chaotically disorganized during chronic unresolved inflammation. This process may promote microenvironment disorganization, eventually leading to cancer onset. The "chaotic" local microenvironment rearrangement induced by the chronic inflammatory reaction prompted HF Dvorak to suggest that tumors are "wounds that do not heal."^[123] This process can be viewed as an abortive attempt to re-establish homeostasis through (abnormal) tissue remodeling, where the survival and transformation of epithelial cells is paradoxically fostered by both cells and microenvironmental components participating in the inflammatory reaction.^[124] Indeed, the onset of several tumors has been associated to chronic, abnormal inflammatory processes.^[30]

A special interest has been aroused by Nitric oxide, IκB kinase,^[125] the upstream kinase that activates NF-κB, TNF-α,^[126] COX-2 proteins,^[127,128] interleukins (IL-1), IL6, and IL-8,^[129] and, consequently, many of these components have been recognized as potential targets for anticancer treatment given that it has long been recognized that by reducing chronic inflammation would lower the risk of developing cancer. Indeed, epidemiological studies have demonstrated that people taking anti-inflammatory drugs have a clear reduction in their risk of developing cancer.^[130,131] In addition, preliminary clinical trials demonstrated a significant benefit in treating cancer patients with synthetic as well as natural anti-inflammatory drugs.^[132,133] Overall, these pharmacological agents do not recognize a single, specific target, given that the multi-faceted activities of the administered drugs (anti-proliferative, angiostatic, antiinflammatory, metabolic activity, immunomodulatory), and their differential cell-specific activities, the exact mechanisms of action of the selected drug combinations are difficult to pin down. Instead, these substances are likely to influence the cell-microenvironment as a whole, displaying both genomic and non-genomic effects.^[134,135]

Paradoxical effects of compounds targeting the microenvironment

Many substances able to target the cancer microenvironment share the uncommon property to act in a paradoxical manner, that is, by exerting opposite effects on different cells or even in different tissues. For instance, bioflavonoids, melatonin and even Vitamin C are well-known to be potent scavenging free radicals agents in normal tissues. Instead, they promote reactive species release in cancerous cells, thus acting as pro-apoptotic factors.^[136-138]

A special case in point is represented by polyphenols extracted from grape and tea, which exert many invaluable anticancer effects. Yet, interest in their clinical use comes from the observation by which such compounds behave as an "intelligent bullet", given that they can influence systems properties by distinguishing in between normal and cancer cells, triggering even opposite effects.^[139] As evidenced for other natural molecules (melatonin,^[140] retinol^[141]), cell proteins (TGF-β) or collagen modifiers (hyaluronidase inhibitors,^[142] Lysyl-oxidase^[143] and Vitamin C^[144,145]), this meaningful feature could likely be ascribed to the different biophysical context which enables the system to differentially respond to a same cue.

Perhaps the best studied example of molecular paradoxes in cancer is represented by TGF-β behavior.^[146] Both anticancer as well as cancer-promoting effects have been alternatively ascribed to TGF-β, until it becomes clear that the "dichotomous" nature of this molecule can be understood only by looking at the tissue-context in which TGF-β is called upon to exert a biological function.^[147] Indeed, tumor stroma not only plays an important role during cancer initiation and progression, but also in determining whether TGF-β suppresses or promotes tumor formation.^[148] That challenging puzzle can be solved by referring data to a higher (tissue) level of observation and by adopting a systems approach. For instance, TGF-β may trigger opposite outputs depending on the tissue stiffness; under mechanically unloaded conditions (floating matrices), TGF-β stimulated contraction directly as an agonist and indirectly by preactivating cells to express the myofibroblast phenotype, whereas, under mechanically loaded conditions (stressed matrices), TGF-β had no direct agonist effect on contraction.^[149] That example highlights how when the cell-microenvironment interactions are kept in consideration, apparently conflicting results end up as such, and paradoxes may likely find a compelling explanation.

Conclusion

For a while, cancer has been considered like a Spinoza's monad, that is, as a microcosm emerging from a deregulated genome occurring in a single cell. Yet, that paradigm has encompassed several shortfalls and it is now increasingly recognized that the overall framework is very much complex that previously thought. We have to "revise the very definition of the term "carcinoma" (currently defined in classical terms as a malignancy derived from epithelial cells), and [...] we need to regard carcinogenesis and carcinomas as phenomena that occur in tissues, not in individual cancer cells. From this perspective, the microenvironment becomes an integral, essential part of the cancer".^[150]

These evidences suggest that physical cues conveyed by stroma or biochemical compounds acting through a pleiotropic-system manner may be eventually more efficient than selective targeted-based drugs and could thereby open new avenues for therapeutic solutions. Conversely, it is now essential to develop new integrated therapeutic strategies focused of targeting the tumor microenvironment, in order to prevent and eventually cure cancer.^[151]

The successful results obtained in treating even few selected cancers through microenvironment-drug modifiers, have

firmly established the paradigm of “differentiation therapy” as a valid approach for the reversion of malignancy in the treatment of neoplasia in human patients. It remains the task to identify newly and more efficient compounds able to globally active in resetting the cell-stroma interactions. That issue can be pursued by combining well-designed pharmacological substances, as well as nutrients and other natural compounds able to selectively target the microenvironment.^[152,153]

A compelling body of evidence suggests that the potential for cancer reversibility exists. However, rigorous experimental characterization of how this process proceeds is necessary before the existing research establishment should take notice and radically change its direction.

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How to cite this article: ???

Source of Support: Nil, **Conflict of Interest:** None declared.