Original Article
Human somatic cells acquire the plasticity to generate embryoid-like metamorphosis via the actin cytoskeleton in injured tissues

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Abstract: Emergent biological responses develop via unknown processes dependent on physical collision. In hypoxia, when the tissue architecture collapses but the geometric core is stable, actin cytoskeleton filament components emerge, revealing a hidden internal order that identifies how each molecule is reassembled into the original mold, using one common connection, i.e., a fractal self-similarity that guides the system from the beginning in reverse metamorphosis, with spontaneous self-assembly of past forms that mimics an embryoid phenotype. We captured this hidden collective filamentous assemblage in progress: Hypoxic deformed cells enter into intercellular collisions, generate migratory ejected filaments, and produce self-assembly of triangular chiral hexagon complexes; this dynamic geometry guides the microenvironment scaffold in which this biological process is incubated, recapitulating embryonic morphogenesis. In all injured tissues, especially in damaged skeletal (striated) muscle cells, visibly hypertrophic intercalated actin-myosin filaments are organized in zebra stripe pattern along the anterior-posterior axis in the interior of the cell, generating cephalic-caudal polarity segmentation, with a high selective level of immunopositivity for Actin, Alpha Skeletal Muscle antibody and for Neuron-Specific Enolase expression of ectodermal differentiation. The function of actin filaments in emergent responses to tissue injury is to reconstitute, reactivate and orchestrate cellular metamorphosis, involving the re-expression of fetal genes, providing evidence of the reverse flow of genetic information within a biological system. The resultant embryoid phenotype emerges as a microscopic fractal template copy of the organization of the whole body, likely allowing the modification and reprogramming of the phenotype of the tumor in which these structures develop, as well as establishing a reverse primordial microscopic mold to collectively re-form cellular building blocks to regenerate injured tissues. Tumorigenesis mimics a self-organizing process of early embryo development. All malignant tumors produce fetal proteins, we now know from which these proteins proceed. Embryoid-like metamorphosis phenomena would represent the anatomical and functional entity of the injury stem cell niche. The sufficiently fast identification, isolation, culture, and expansion of these self-organized structures or genetically derived products could, in our opinion, be used to develop new therapeutic strategies against cancer and in regenerative medicine.

Keywords: Hypoxia, cancer, intercellular collisions, actin-myosin filaments, embryoid-like metamorphosis

Introduction

All systems whose particles are in continuous dynamic movement submit to the laws of physics. This means that throughout the life space-time interval of any system, there is the absolute potential to enter into a collision state at any given moment. Cancer is an excellent ex-ample of a biological collision model for studying chaotic systems. It is also the most spectacular biological time machine of information reversal, and in an activated collective memory of ancestral genes, it carries data linking the origin of cancer to the origin of life. The developmental processes of primordial patterning, in a general replication of past forms, order arises

naturally from chaotic systems, with self-organized behavior emerging near critical transitions [1].

In our preliminary study, we described and documented the self-assembly of actin filament geometric triangular chiral hexagon complexes (GTCHC) in human pathologic tissues at macroscopic and microscopic levels, mainly in cancer tissue injury processes [2]. The genesis of these complexes occurs through intercellular collisions that produce degradation of ejected actin filaments in the form of spin-domain interactions, that is, pairs of filaments with left- and right-hand sub-patterns of spin spirals which are associated with the orientation of the spinning process, which can be an expansion or contraction disposition in the interphase matrix. Actin filament units describe trails of uniform spatial translations, interconnections, and communications in the interstitial microenvironment.

On the premise that behind the GTCHC filamentary complexes there must be a corresponding vasoactive vascular order, we decided to rigorously review macroscopic and microscopic material from the malignant tumors in which GTCHC complexes were identified, and we coupled that approach with analysis of the Von Willebrand Factor VIII-related antigen. We have detected macroscopic spatial assembly integration between the cut surfaces on the ventral parts of the tumors where the GTCHC complexes were located, with the deep tumor dorsal bed areas containing an organized vascular net. This organization is configured in a geometric scaffold of separate bipolar triangular chiral mirror filaments and vasoactive vessels linked by a helicoid pattern.

Why did nature develop such a complex organization of intrinsic structure in hypoxically injured tissues? We were only able to understand the purpose of these structures after 5 years of methodological observations. When the tissue architecture collapses, but the geometric core is stable, actin-filament components emerge, revealing a hidden interior that identifies how each molecule is reassembled into the original mold, using one common connection, i.e., a fractal self-similarity that guided the system from the beginning, in reverse metamorphosis. GTCHC complexes serve as guides in the orga-

nization of the chaotic neovascularization in the hypoxic tissue that results from injury, in the assembly of the vascular niche organization following a predetermined invariant geometric attractor pattern. The platform of domain interactions consisting of separate triangular chiral filaments that are linked by spiral helicoid patterns produces from this niche a master-built construction with a differentiated ectoderm embryoid-like structure and showing immunopositivity for Neuron-Specific Enolase [3-5].

Tissue injury can generate induced stem-cell niches, which are defined as cellular microenvironments that are produced during tissue injury, are triggered by injury and the local responses of support cells, and enable the possibility of repair by endogenous or transplanted stem cells. These environments have been demonstrated in several injury models, most notably in the CNS. The term "induced stem-cell niche" was coined by Jaime Imitola and Evan Y. Snyder when they demonstrated that during stroke, astrocytes and endothelial cells are able to create a permissive environment for neural regeneration, and they observed that neural stem cells (NSCs) migrate through the parenchyma along nonstereotypical routes in a precisely directed manner across great distances to injury sites in the CNS, where they might interact with niches harboring local, transiently expressed reparative signals [6].

During injury, inflammation may be viewed not simply as playing an adverse role but also as providing stimuli that recruit cells with a capacity to promote regenerative homeostasis. Multicellular organisms show adaptive reactions for their survival when they are exposed to an atmosphere with reduced oxygen concentration. We therefore addressed the question of whether hypoxia by itself could generate the formation of these structures in tissues not related to tumor conditions and if, in acute tissue injuries, we can predict the formation of these filament patterns.

Materials and methods

This study was approved by the ethical subcommittee of the Faculty of Medicine, University Cooperative of Colombia, and it followed the guidelines of the Minister of Health No. 8430 of 1993, in accordance with the Declaration of

Table1. Relationship between embryoid like Metamorphosis (ELM) and Neuron-Enolase Immunopositivity

		Embryoid Metamorphosis	
		Positive	Negative
Enolase Stain	Positive	45	10
ENO2	Negative	1	4

Table 2. Relationship between Embryoid like Metamorphosis (ELM) and geometric Actin filaments Immunopositivity

		Embryoid Metamorphosis	
		Positive	Negative
Actin-alfa	Positive	47	3
inmmunostain	Negative	3	7

Helsinki. All patients had signed an informed consent form for the use of their biological materials for diagnostic and research purposes.

From the Department of Pathology, Departmental Hospital of Villavicencio, we collected sixty (60) surgical specimens from tissues with severe acute injuries. Mesodermal origin: Twenty (20) trauma lower-limb amputations from accidents to soldiers affected by antipersonnel mines, twenty (20) no-trauma lower-limb amputations related to vascular diabetes and other diseases. Endodermal origin: Five (5) acute injury surgical kidney specimens for trauma, five (5) acute injury surgical pancreas specimens from abdominal gunshot. Maternal-Fetal component origin: Ten (10) placentas with severe hypoxia. Representative macroscopic samples of injured tissues in the interface of bleeding necrotic hypoxic zones were taken and histologically documented using routine hematoxylin and eosin staining. Areas or paraffin blocks containing more embryoid structures were identified and selected for Actin, Alpha Skeletal Muscle antibody and for Neuron-Specific Enolase immunostaining.

Samples were selected from traumatic and non-traumatic tissues showing clear evidence of decreased distal vascular supply and the following clinical signs: peripheral cyanosis, loss of deep and superficial sensitivity, and transcutaneous oxygen saturation < 40%. In cases of traumatic tissues, major trauma signs of arte-rial injury and open fractures were selected; for non-traumatic injury, Doppler studies have reported approximately 72% artery stenosis, mostly from patients with type II diabetes mellitus and microscopic and macroscopic apparent arterial disease. The placentas were selected mainly due to secondary hypoxia: placental insufficiency, calcifications of chorionic villi (in pregnancies with more than 40 weeks of gestation), placental abruption, retro-placental hematoma, and vasa previa.

We chose exceptional cases of penetrating abdominal trauma, specifically gunshots to intra-abdominal organs such as the pancreas and kidney; after surgical intervention, partial pancreatectomies and total nephrectomies were performed in these patients. Respective tissue samples were taken for histopathological and immunohistochemistry analysis.

Blood immunostaining

To verify the histogenesis of spiral/helicoid framework-related GTCHC complexes, we performed immunostaining to study the distribution, localization and immunoreactivity of von Willebrand Factor VIII-related antigen. Sixty formalin-fixed and paraffin-embedded tissue sections with the most representative hot-spot geometric areas were analyzed. We performed immunohistochemistry using a standard protocol for paraffin sections (3). Samples were scored as ni (no immunostaining), low (≤10% immunopositivity cells), or high (>10% immunoreactivity cells).

Actin immunostaining

We used Anti-Alpha Skeletal Muscle Actin antibody- F-actin cross-linking protein; Alpha-Alpha actinins belong to the spectrin gene superfamily which represents a diverse group of cytoskeletal proteins, including the alpha and beta spectrins and dystrophins. Alpha actinin is an actin-binding protein with multiple roles in different cell types. In nonmuscle cells, the cytoskeletal isoform is found along microfilament bundles and adherens-type junctions, where it is involved in binding actin to the membrane.

Sixty formalin-fixed and paraffin-embedded tissue sections with the most representative hot-spot geometric filaments interface-embry-
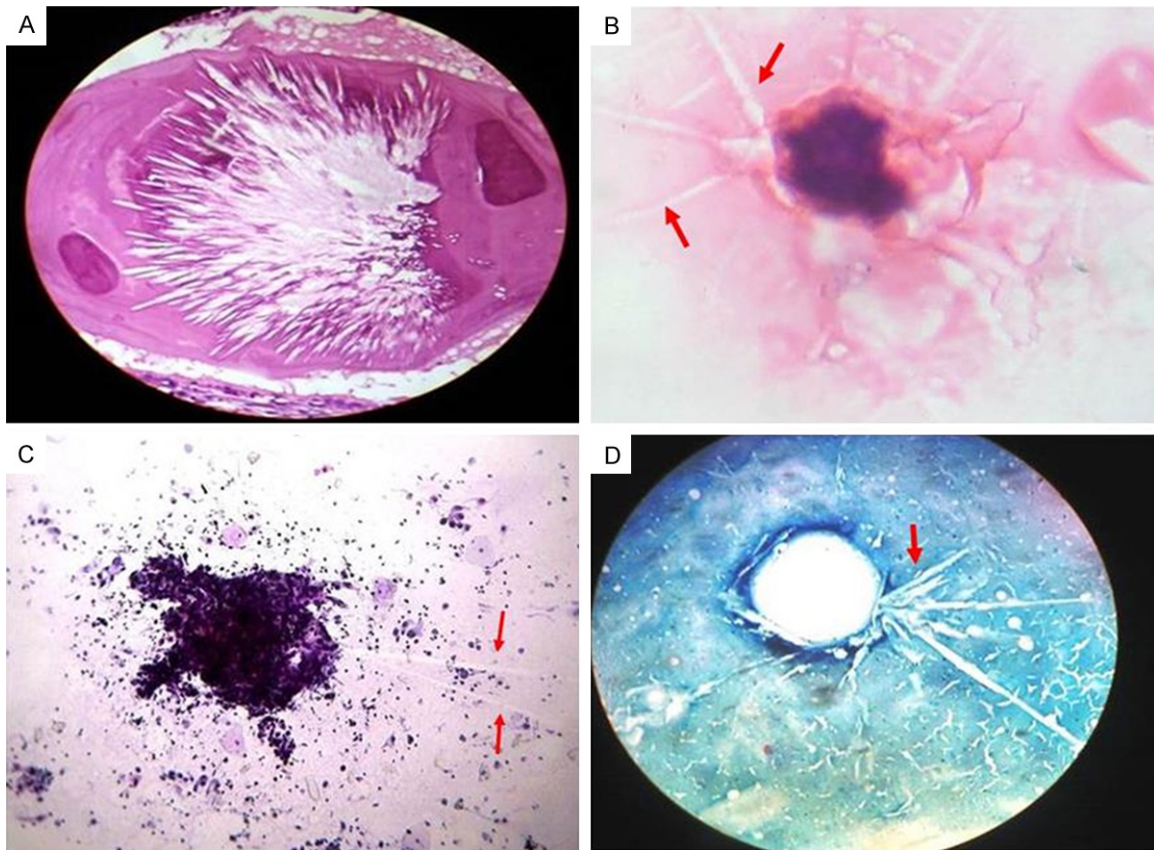
Figure 1. Embryoid like metamorphosis dynamic sequential process: Ejected migratory actin cytoskeleton filaments identified into intercellular collisions. A. Prostate adenocarcinoma (H.E stain 40x). B. Malignant ascitic fluid. (Papanicolaou stain 20 x). C, D. Cervical adenocarcinoma, (Papanicolaou stain 20x) cervical smears.

oid-like pattern areas were analyzed using Anti-Alpha Skeletal Muscle Actin antibody. We performed immunohistochemistry using a standard protocol for paraffin sections. The scoring was performed as follows: Ni (no immunostaining), low (≤10% immunopositivity cells), or high (>10% immunoreactive cells).

Neural immunostaining

We used used enolase 2 (NSE, gamma neuronal), also known as ENO2. This isoenzyme is a homodimer found in mature neurons and cells originating from the neural ectoderm.

Sixty formalin-fixed and paraffin-embedded tissue sections with the most representative hotspot embryoid-like pattern areas were analyzed using an antibody against neuron-specific enolase as a marker. We performed immunohistochemistry using a standard protocol for paraffin sections. The scoring was performed as follows: Ni (no immunostaining), low (≤10% im-

munopositivity cells), or high (>10% immunoreactive cells).

Pathology evaluation method

Based on the observational study, diagnosis, and analysis of more than 10,000 surgical specimens and biopsies corresponding to inflammatory processes, malignant tumors, and benign lesions during the last 5 years, we have established a pattern-recognition algorithm that allowed us to identify self-assembling structures not displayed previously, thanks to the relative frequency of occurrence of these structures in a number n of repetitions in a space-time generated spontaneously without any mediating experiment.

Cellular pleomorphism, cells that grow in single rows, and irregular nuclei, cells, glands, acini, and angulation are common morphological findings in cancer tissues, features that pathologists have simply described, not explained.
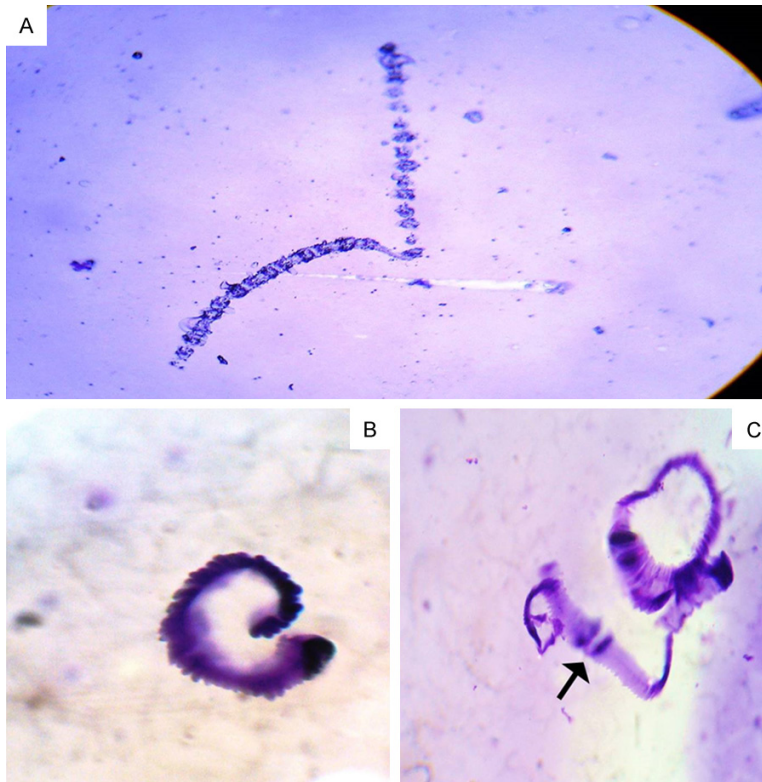
Figure 2. Embryoid like metamorphosis dynamic sequential process: A. Thick actin cytoskeleton filament "jumps" over thin filament avoiding collision. In contact Inhibition of locomotion, when two filaments collide they attempt to move in a different direction to avoid future collisions. B, C. Filamentary chiral spin-spiraled currents with a gyrus in opposite directions, observe in C visible hypertrophic intercalate actin cytoskeleton filaments (arrow), organized along anterior-posterior axis, generating cephalic-caudal polarity. Material captured from Ovarian adenocarcinoma ascitic fluid (Papanicolaou stain 20x).

We have observed and documented how the generator of this "linear order" is given by the degradation of actin cytoskeleton filaments that adopt positions in the nuclear and cytoplasmic membranes and deform the cell, generating angulation and geometric GTCHC complexes. We are decoding the underlying connection between geometry and embryoid-like metamorphosis. A frequentist repeatable-event approach established that this geometrical order is the environmental platform in which 5 to 6 hyperchromatic cells fuse under conditions of tissue injury or hypoxic disadvantage, generating structures with cephalic-caudal segmentation mediated by actin filaments. Histological sections from extreme tissue injury where these structures emerge were isolated from skeletal muscle, which derives from mesoderm, and from pancreatic and renal tissue, which derive from endoderm, and the respective paraffin blocks were analyzed using immunohistochemistry for neuron-specific enolase to evaluate neuro ectoderm differentiation.

We determined the relationship between embryoid-like metamorphosis (ELM) and vascular niche geometric attractor transformation areas using neuron-specific enolase antibody immunopositivity; Chi-squares for the proportions were estimated using EPI-INFO software (version 7.0, Centers for Disease Control and Prevention, Atlanta, GA).

Statistical analysis

Statistical analysis determined that 60 hypoxic tissues had high immunopositivity for neuron-specific enolase, with the presence of ELM (embryoid-like metamorphosis) in 45 cases (75%, χ^2 = 17.16, P = 0.000034), which was negative in 1 case (1.6%), as shown in **Table 1**. The relationship between enolase and ELM was based on the chi-square test, which yielded a chi-squared value of 9.7911 (df = 1, *P* value < 0.001754, and an alpha of 0.01; the null hypothesis was rejected). Localization of invariant geometric actin filaments in the vascular niche is related to the tissues of the surrounding ELM in which the structures are located; in 47 cases, the location was associated with geometric pattern (78.3%), and the association was negative in 3 cases (5%). According to the null hypothesis, the variables are dependent based on the chi-square test, which yielded a chi-squared value of 24.576 (df = 1, *P* value of 1E-06, significant at p < 0.01) (**Table 2**).

Results

Here, we use step-by-step photographic evidence to recapitulate this hidden sequential biological transformation from primary intercel-
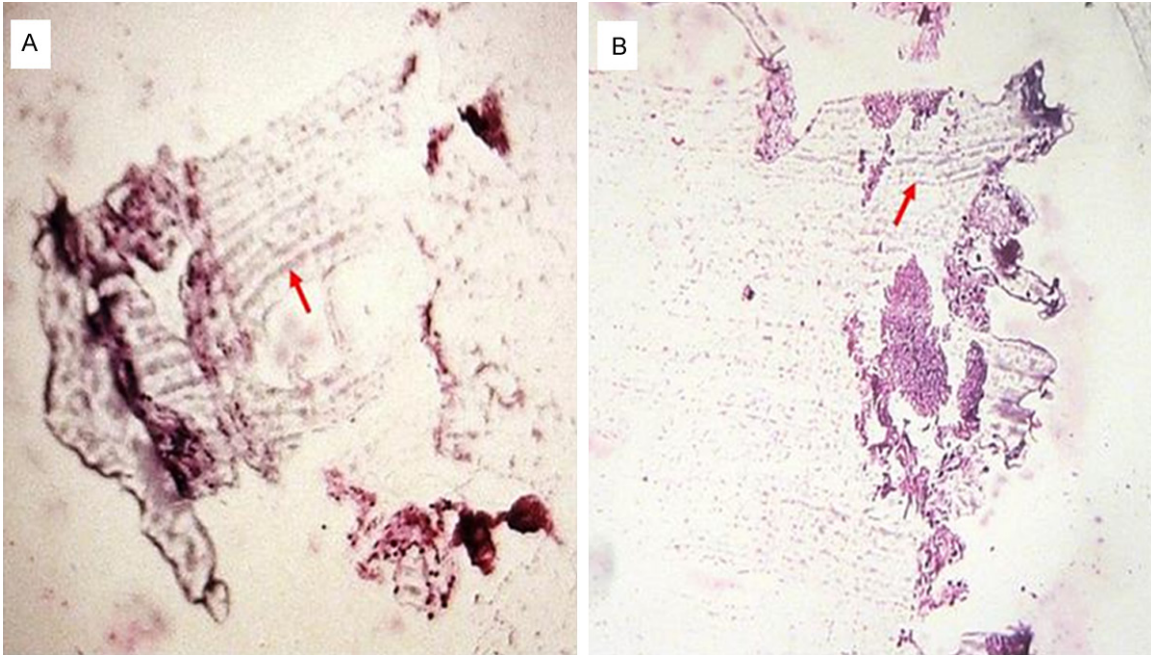
Figure 3. A, B. Photomicrographs shows Actin-Alfa Skeletal Muscle immunoposivity of collective self-assembly filamentous structures organized in zebra stripe pattern (arrows). Material captured from tissue hypoxic and necrotic areas.
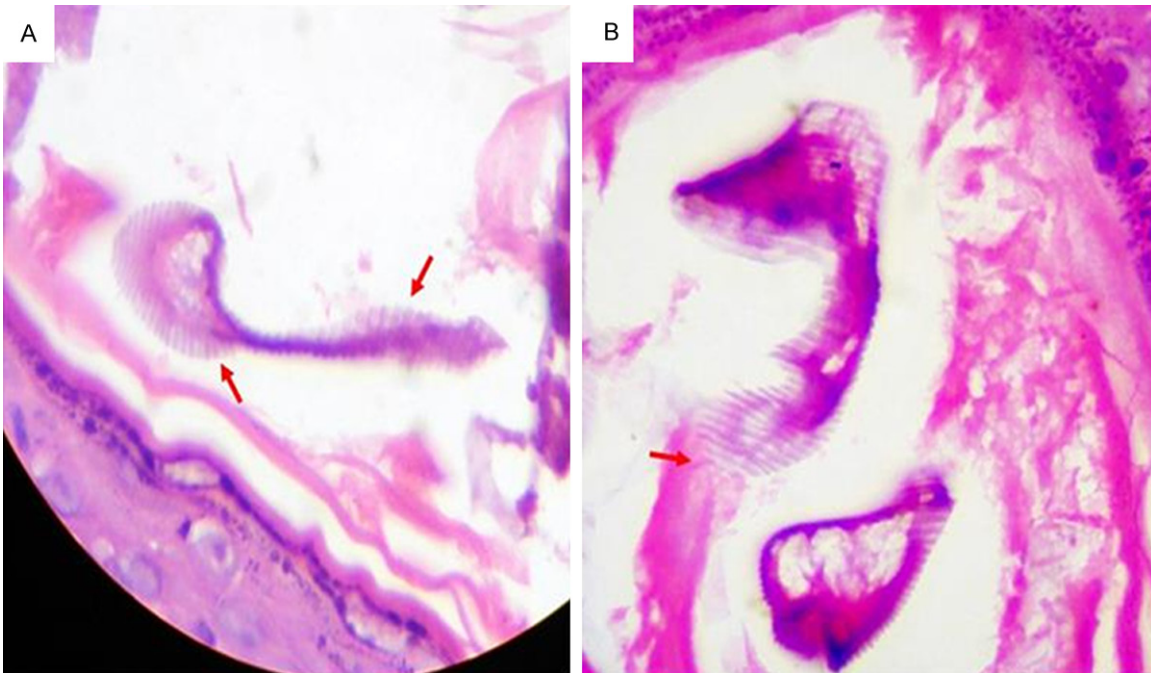

Figure 4. A. Image illustrates sequential embryoid like metamorphosis step 1: Observe the primitive actin filamentous polar structure with distinctive zebra patterning (arrow) Material was captured from malignant Pilar tumor. (H.E stain 40x). B. Sequential embryoid like metamorphosis step 2: Specific transformation zone (arrow). Observe how visible intercalate-parallel hypertrophic actin cytoskeleton filaments generate sea horse cephalic-caudal embryoid like phenotype segmentation. Material was captured from malignant Pilar tumor. (H.E stain 40x).

lular collision events to the generation of geometric triangular chiral actin filaments that guide the metamorphic changes of cells under extreme injury.
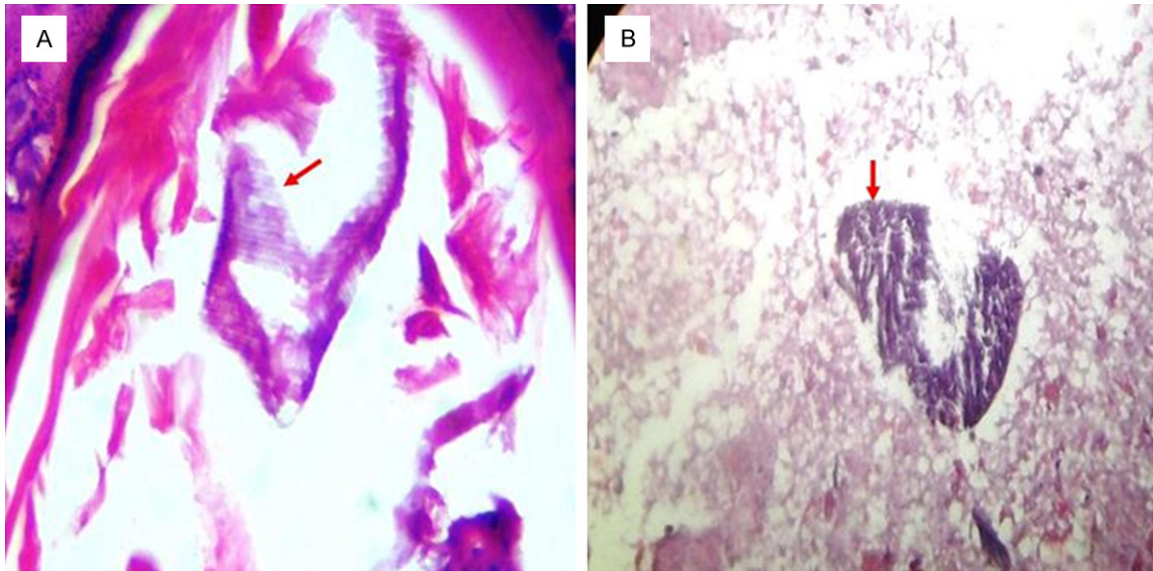
Figure 5. A. Sequential embryoid like metamorphosis step 3: Observe how visible intercalate-parallel hypertrophic actin cytoskeleton filaments generate cephalic-caudal embryoid like phenotype segmentation. Material was captured from malignant Pilar tumor. (H.E stain 40x). B. Actin embryoid like metamorphosis immunopositivity with zebra stripe patterning. Material was captured from necrotic inflammatory zone.
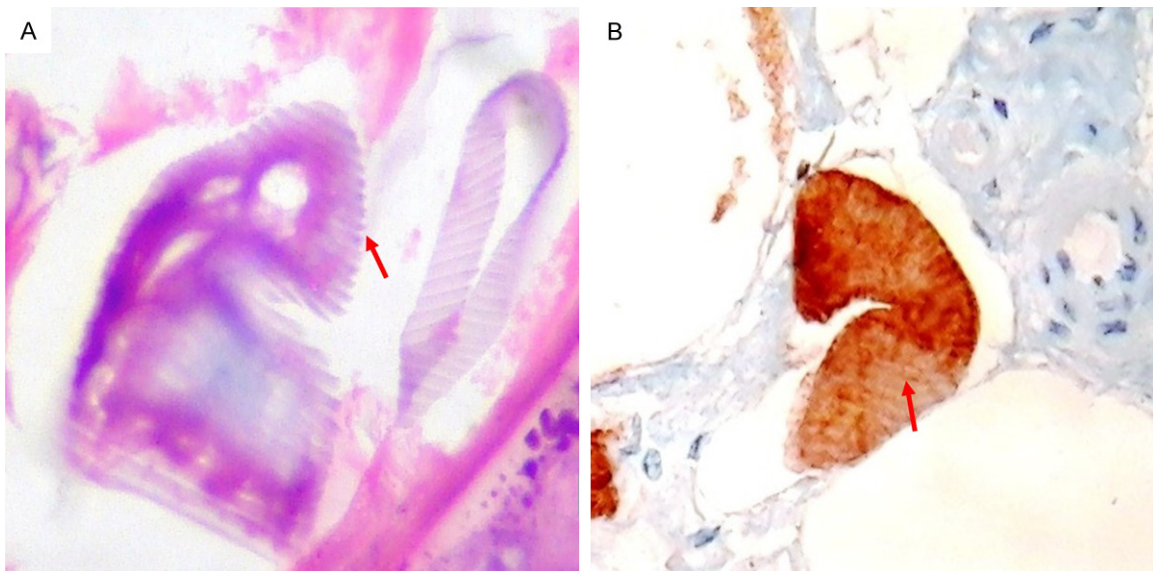

Figure 6. A. Sequential embryoid like metamorphosis step 4: Hypertrophic Intercalate-parallel actin cytoskeleton filaments (arrow) are organized along anterior-posterior axis generating perfect well defined spontaneous self-assembly structure that mimic embryoid like phenotype. Microphotograph captured from malignant Pilar tumor (H.E stain 40x). B. Actin embryoid like metamorphosis immunopositivity with zebra stripe patterning (arrow). Material was captured from necrotic inflammatory zone.

We identified and documented geometric triangular chiral structures intimately associated with cancer and acute extreme tissue injury that result in spontaneous self-assembly of past forms that mimic an embryo-like phenotype. We captured this hidden collective filamentous assemblage in progress: Hypoxic deformed cells enter intercellular collisions, generate migratory ejected filaments, and produce self-assembly of hexagon triangular chiral complexes. This dynamic geometry guides the micro environmental scaffold in which it is incubated, inducing this biological process that recapitulates embryonic morphogenesis. In all
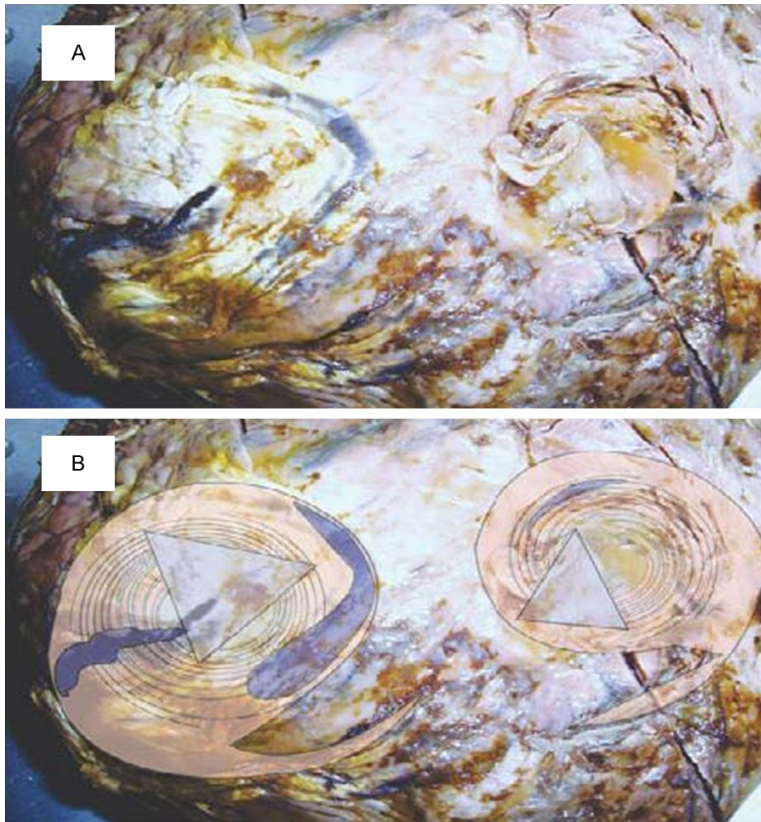
Figure 7. Macroscopic chiral spin current filament with collider partners dipole vascular behavior. A. Macroscopic dorsal view of leiomyosarcoma. Collider partners' pair of spiral filaments that are oriented in chiral opposed currents. Observe how in the left image the vascular network follows the spiraled pattern with ectasia and vasodilatation. Contrary, Image on the right show vasoconstriction. In the center of the spirals appear triangular mirror images on each side. B. Spatial organization of image in A.

injured tissues, but especially in damaged skeletal (striated) muscle cells, visibly hypertrophic intercalate actin-myosin filaments are organized in zebra pattern along the anterior-posterior axis in the interior of the cell, generating cephalic-caudal polarity segmentation, with a high selective level of immunopositivity for actin alfa skeletal muscle antibody and for neuron-specific enolase expression of ectodermal differentiation. The function of cytoskeletal filaments in emergent tissue injury responses is to reconstitute reactivate and orchestrate cellular metamorphosis and to induce the re-expression of fetal genes in evidence of the reverse flow of genetic information within a biological system.

Initiator phase

This phase begins from the moment at which thousands of malignant, hypoxic cellular membranes embark on the gradual process of degeneration with increasing collisions (**Figure 1**). Tissue hypoxia generates intercellular collisions of deformed hypoxic cells, and these collisions dynamically structure filamentary chiral spin currents that can lead to expansion or contraction (**Figure 2**). Characteristic zebra stripe pattern of actin immunopositivity intercalated parallel filaments order organization was identify in tissue injury and tumor necrotic areas (**Figure 3**). Visibly hypertrophic intercalated actin filaments are organized along the anterior-posterior axis in the interior of the cell, generating embryoid-like sequential steps metamorphosis (**Figures 4-6**).

Generator phase

Chiral spin currents incorporated into the system by actin filaments produce an invariant geometric attractor that guides the chaotic hypoxic vasculature and generates niches of synchronic vasoconstriction/vasodilation functional units. Colliding pairs of spirals that are oriented in opposite directions. These ejected filament particles then split into two components that follow opposite chiral spin currents directions in a helicoid flow pattern (**Figure 7**).

Transformation phase

Vascular niches of synchronic vasoconstriction and vasodilation functional units produce fractal resonances, which are the convergent and confluent phases of the system Complex molecular matter is then organized into the fractal form. As more and more layers of fractal filaments are incorporated into the system, it becomes more complex until it reaches the point of the final master construction, generating embryoid cephalic-caudal polarity segmentation Embryoid-like metamorphosis is the ultimate expression of the interaction of all
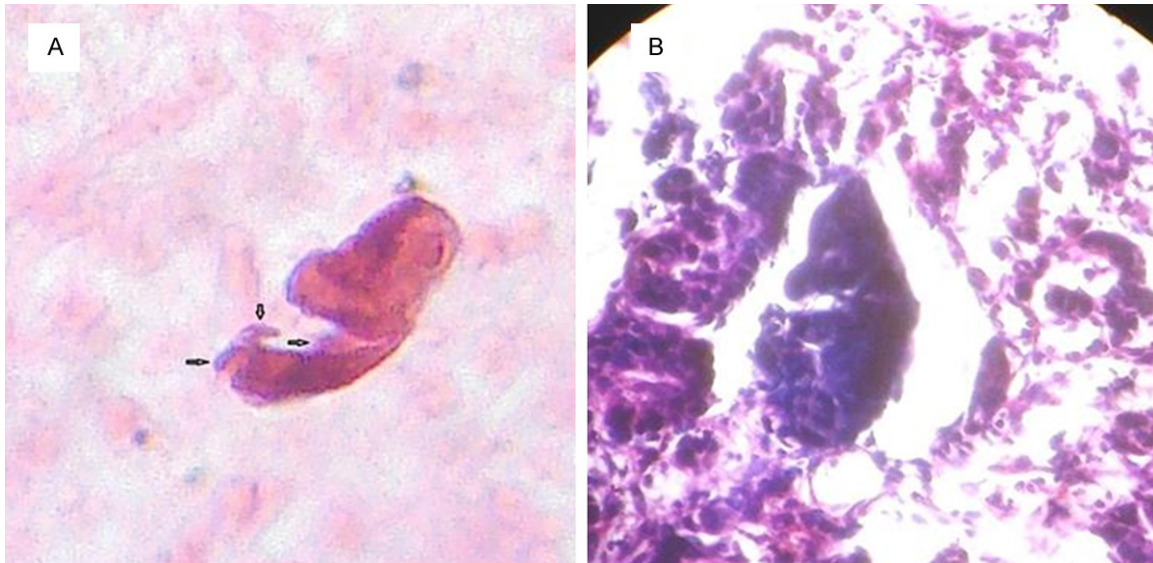
Figure 8. A. Perfect well defined embryoid like structure. Observe head and tail polarity with primitive design of upper and lower extremities (arrows). Material was captured from malignant tumor necrosis area (H.E stain 40x). B. Well defined embryoid like structure within a cavity. Material was captured from gastric cancer (H.E stain 40x).

filamentary forces (**Figure 8**). The pattern or structure is clearly the result of convergent extraction or information exchange (**Figure 9**).

Vascular immunostaining

All 60 tissue sections showed positive immunolabeling. In 47 immunostained sections, geometric structure poles showed opposing immunoreactivity behavior. Vascular niche organization consisting of spatial separate triangular mirror images linked by helicoid patterns also showed opposing immunoreactivity behavior. Vascular contraction lumens showed high immunoreactivity, while mirror triangular vascular dilatation lumens in the opposite pole show no or low immunopositivity of the antibody (**Figure 10**).

Differentiation phase

Differentiation accelerates as the complexity grows. We observed a highly selective distribution of immunostaining with Actin-Alfa Skeletal Muscle and Neural antibody respectively related to clusters of geometric triangular chiral mirror somatic cells, which were organized in an assemblage with an embryoid-body-like phenotype (**Figures 11-13**). Immunostaining consistently revealed individual cells showing neuro-ectodermal differentiation in the context of the whole hypoxic tissue.

Comparative verification

A fingerprint is only valuable when you have a pattern to compare it with. Based on the fact that vertebrate embryos are similar to each other during early development, we compare the structures identified with the most recent real images of Three-Dimensional Analysis of Vascular Development in the Mouse Embryo Atlas [7].

By decomposing these images, verification, as the sequential construction of these embryos, is provided by the fractal accumulation of vascular niches of spatial order, organized by triangular chiral mirror filamentary patterns, linked by the helicoid substrate, which is a constant replication of the pattern. The fractal sum of the sequence order of the filaments results in a spontaneous self-assembly of the vascular structures with one final product: the embryo (**Figure 14**).

Final observational findings

The phenomenon that we documented here represents, to us, the activation of a normal human ancestral biological, physiological cellular adaptation process, a phenomenon that we call embryoid-like metamorphosis (ELM), an ancestral phenotype fingerprint pattern adaptation in response to the tolerance of hypoxia-
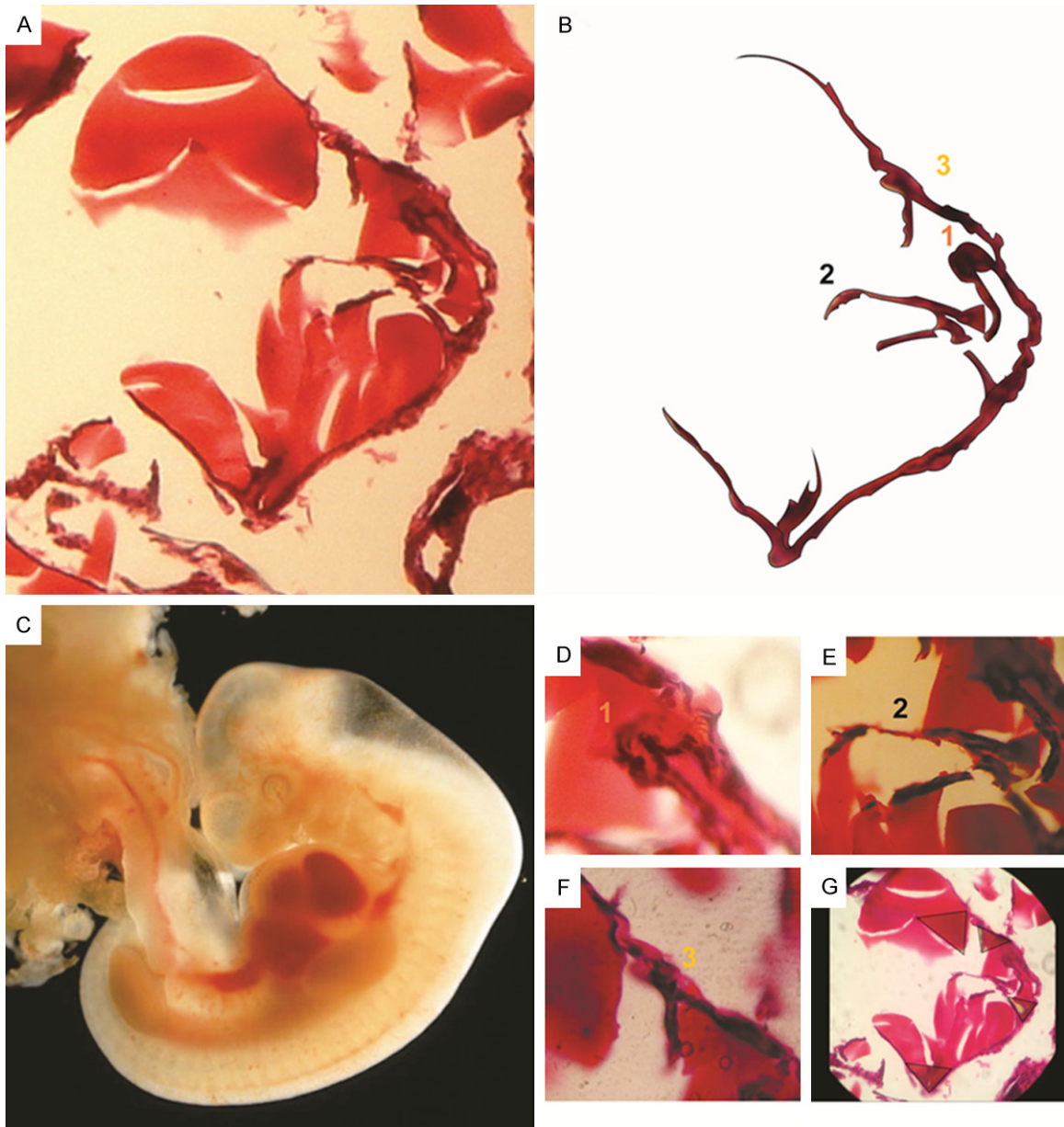
Figure 9. (A) Unique visible embryoid like structure, we are able to visualize their primordial vessel development. The image has sufficient resolution to visualize the finest vessel structures. (B) Detachment to visualize the complete vasculature of the embryo as shown in image (A). (C) 28 days. Embryo comparative image pattern. (D, E) Zoomed in situ to give a magnified view of structures of interest as in image (A) such as 1) Primordial cardiovascular system 2) Vessels connecting the embryonic extraembryonic circulation 3) Cephalic plexus. (scheme). (F) Shows Cephalic plexus in situ. (G) Shows how the embryoid likes structure its build in basis triangular chiral platform. Material captured from Thyroid cell hurtle carcinoma (H.E stain 40X).

protection through the reconfiguration of underlying cellular and subcellular components.

We documented the microscopic cellular embryoid-like metamorphosis in regions of chronic tissue injury and hypoxia corresponding to zones of active bleeding, tumor necrosis, and neovascularization in all types cancer tissues, including carcinomas and sarcomas (Figure S1). We were able to document complete embryoid-like metamorphosis transformation using surgical specimens from in vivo macroscopic tumors (Figure S1U, S1V). We observed clusters of cells organized in an embryoid body
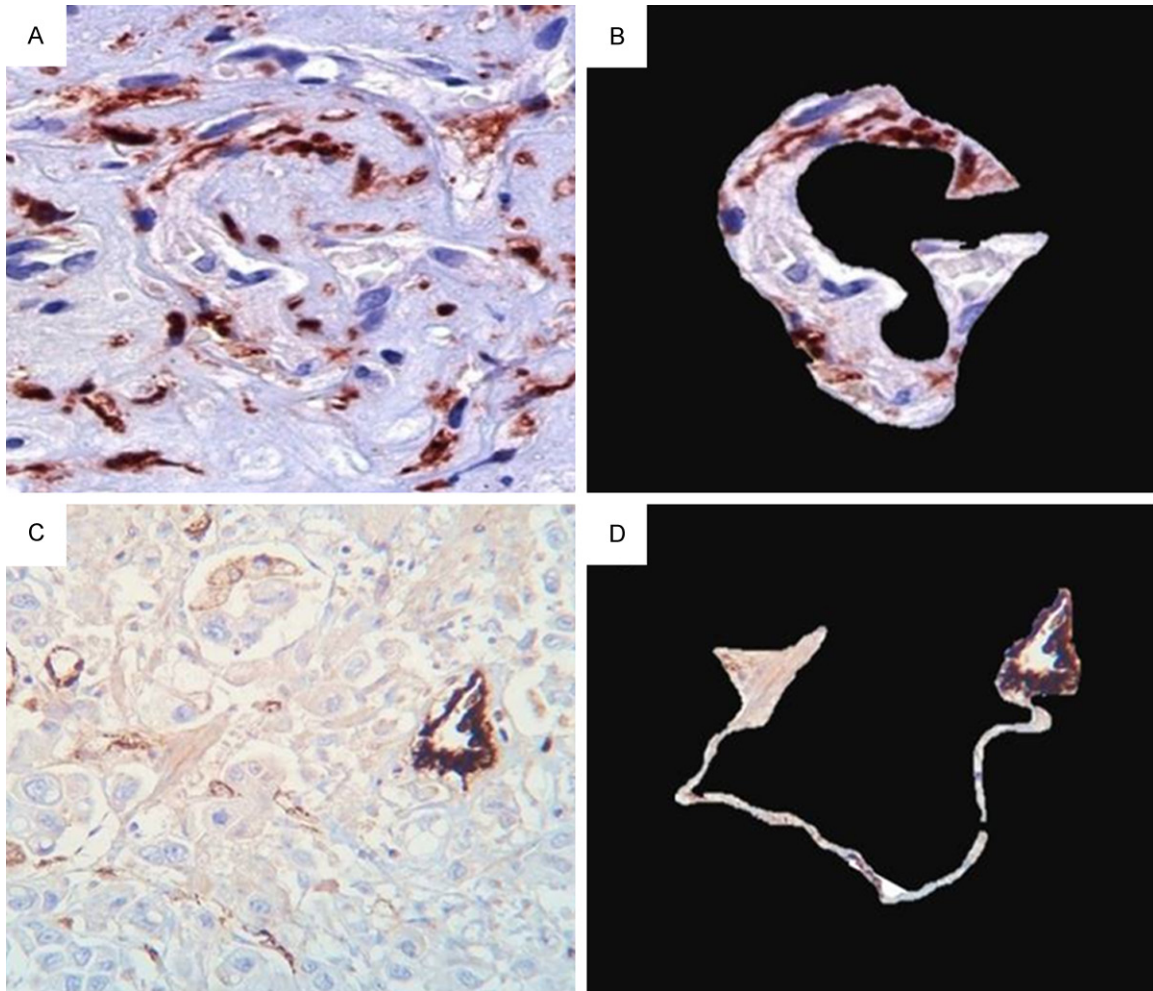
Figure 10. Blood vessels immunostain Factor VIII antibody. (A) Breast carcinoma. Asymmetric polar activity of factor VIII. Triangular vascular contraction lumen show high immunoreactivity. Triangular mirror vascular dilatation lumen in the opposite pole show complete absence of the antibody (40x). (B) Schematic spatial organization of image in (A). (C) Gastric carcinoma Triangular vascular mirror images linked by helicoidal framework. Observe polar immunoreactivity (40x). (D) Schematic spatial organization of image (C).
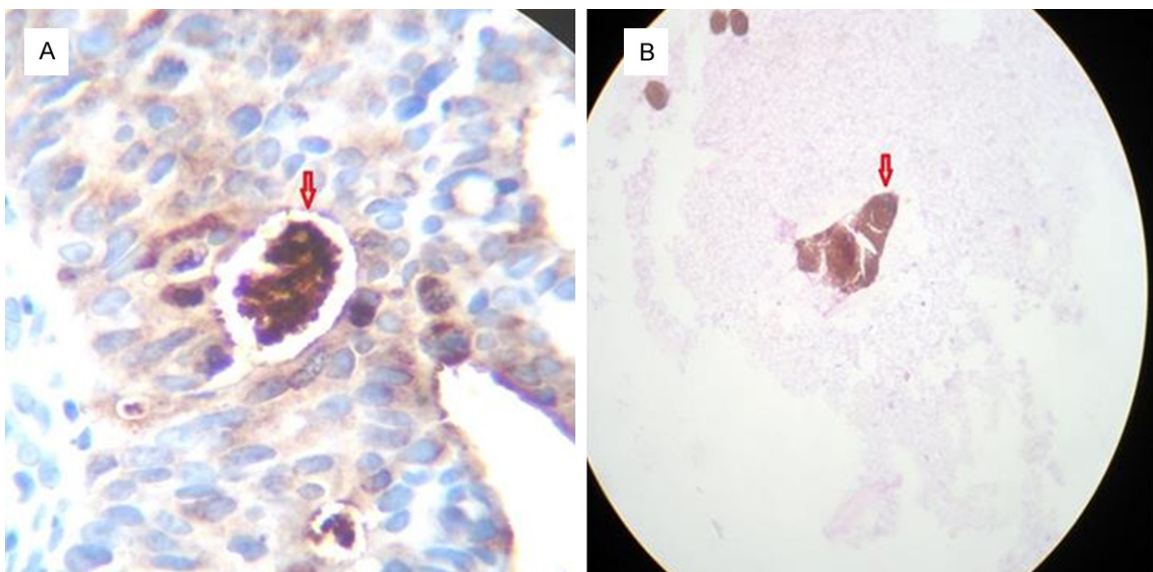
Figure 11. A. Actin embryoid like structure high selective immunopositivity (arrow). Material was captured from Lung squamous cellular cancer (H.E stain 40x). B. Actin embryoid like structure high selective immunopositivity (arrow). Material was captured from tumor necrosis area.
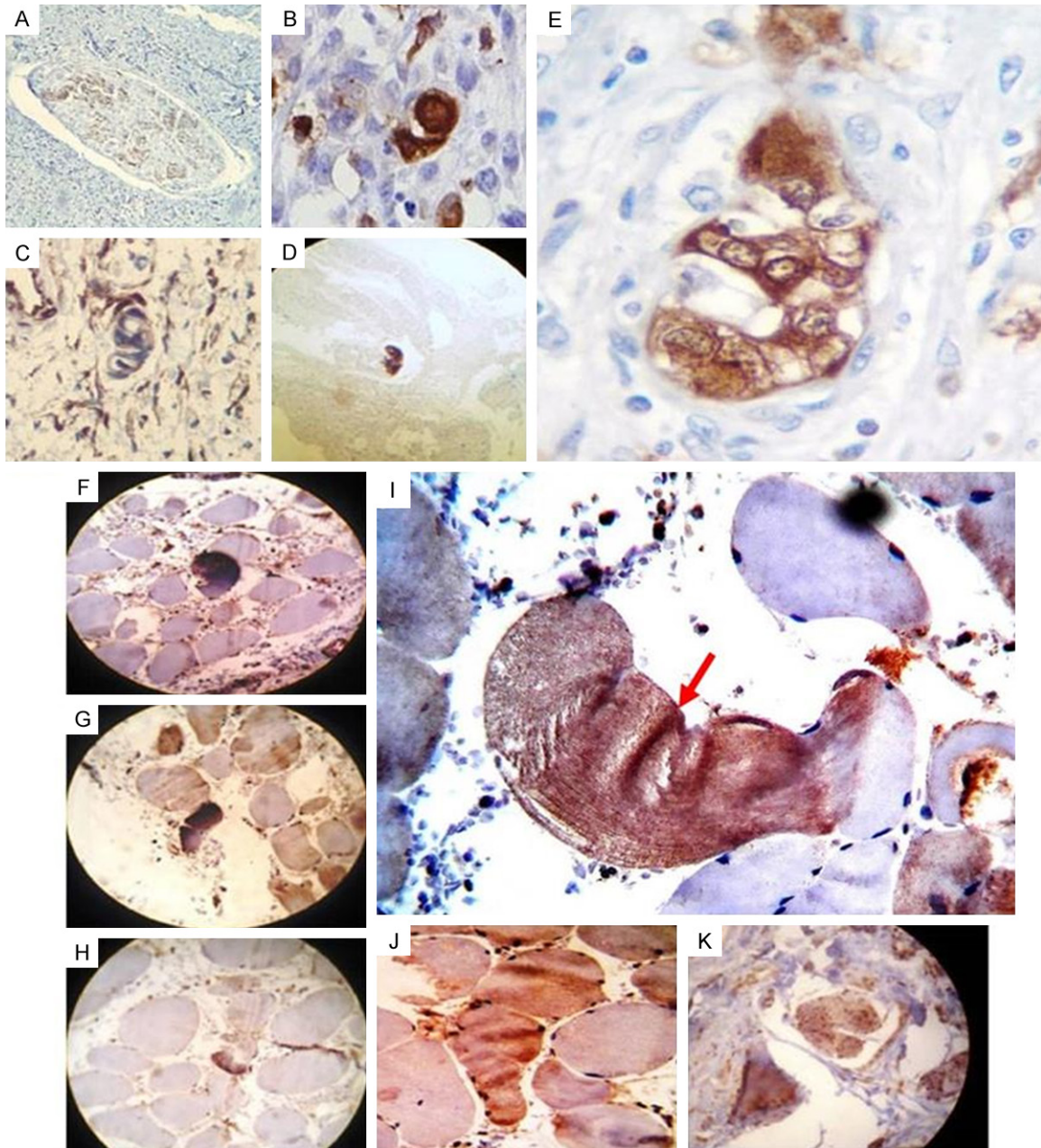

Figure 12. Neuron-specific enolase Immunostaining analysis. Photomicrographs reveal highly selective ectodermal Neuron-specific Enolase immunopositivity of embryoid like structures in response to acute hypoxic tissue damage. (A) Shows Neuron-specific Enolase Immunostaining of control tissue from a peripheral nerve. (B) Lung cancer. (C) Sarcoma tumor. (D) Undifferentiated tumor necrosis area. (E) Micrograph revealing high enolase immunopositivity neuro ectoderm differentiation in a well-defined self-assembly embryoid like structure pattern from a case of prostate carcinoma, observe brown-white zebra striped pattern along anterior-posterior axis. (F-I) Visible hypertrophic actin-myosin filaments with formation of zebra-striped pattern along anterior-posterior axis, phenomena restricted to skeletal muscle cells in transit to embryoid like metamorphosis. Material captured from skeletal muscle in response to acute hypoxic tissue damage traumatic amputation. Observe in image (I). Hypertrophic actin filaments zebra-stripe pattern (arrow) along anterior-posterior axis; from this pattern profile emerge the future segmentation of the embryoid like structure. (J, K) Embryoid NSE positive zebra-striped pattern identified in skeletal muscle extreme injured tissue. (A-D 20x); (E 40x); (F-H 20x); (I 60x); (J, K 20x).
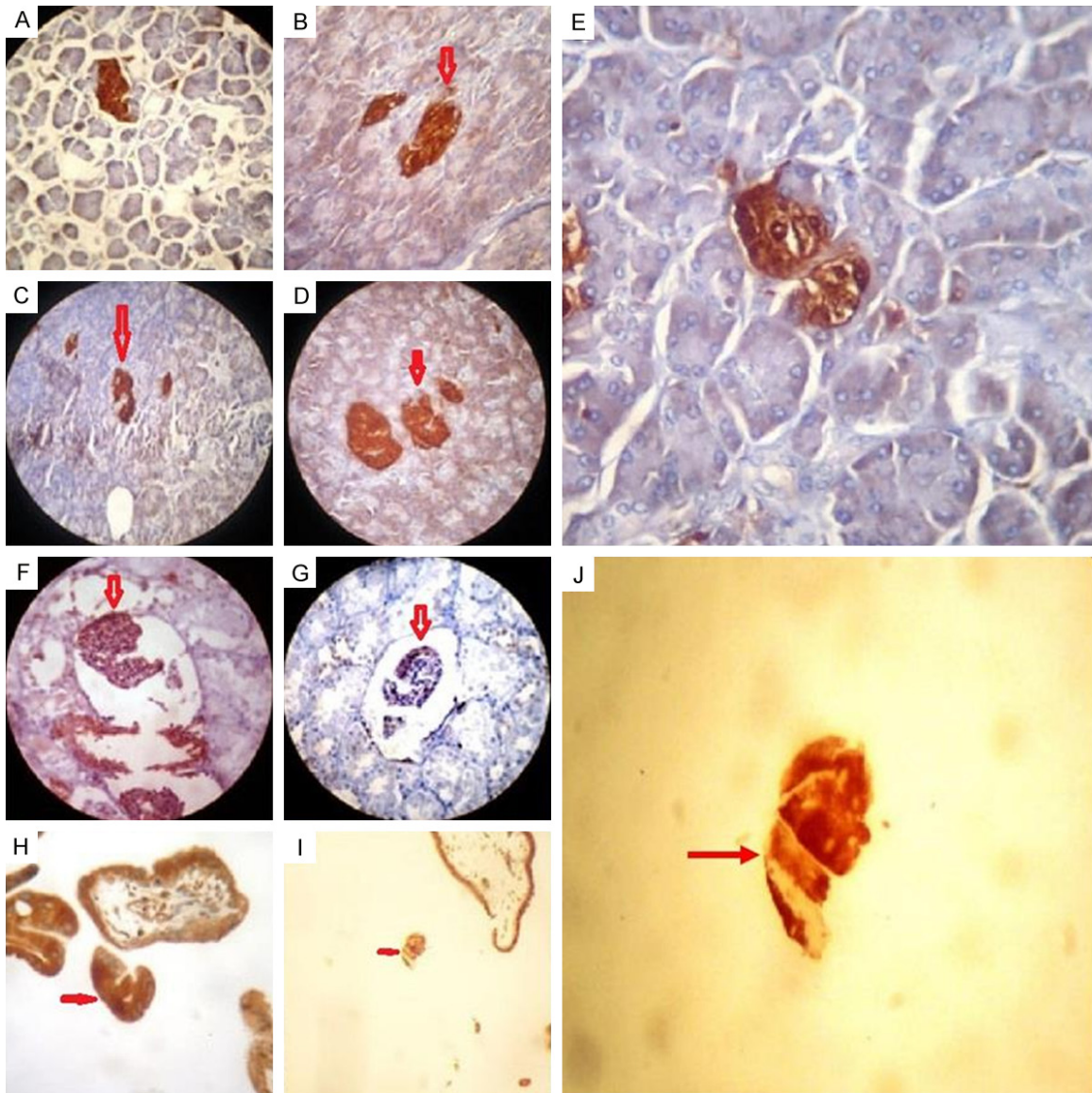
Figure 13. Photomicrographs reveal highly selective enolase immunopositivity of embryoid like structures in response to acute hypoxic tissue damage. (A-E) Selective NSE immune positivity stain neuro ectoderm differentiation of fetal embryoid structures identified in pancreas acute hypoxic tissue damage. (F, G) Selective NSE immune positivity stain of embryoid like structures identified in renal glomerulus acute injured hypoxic tissue damage. (H-J) Selective NSE immune positivity stain of embryoid like structures identified in Placental acute hypoxia Observe in (J). Well-defined NSE positive embryoid, with actin cytoskeleton filament zebra-stripe pattern along anterior-posterior axis. (arrows) (A-D 20 x); (E 40x); (F-I 20x); (J 40x).

phenotype assemblage, in all of the examined tissues skeletal muscle, pancreatic, renal, and placental tissue (Figures S2, S3).

Phenotype characterization

We identified a unique "fingerprint" morphological sequence formation in these embryoid transformations. Clusters of somatic cells assemble in a unified pattern with zebra-striped filaments along the anterior-posterior axis that controls spatial-temporal development. From this template of segmentation patterning, polar-body-like structures emerge. The complete embryoid body structure is the final result of cell fusion and accumulation of fractal copies of vasoactive vessels, which is clearly visible in hematoxylin-eosin staining and in positive immunostaining for neuron-specific enolase.
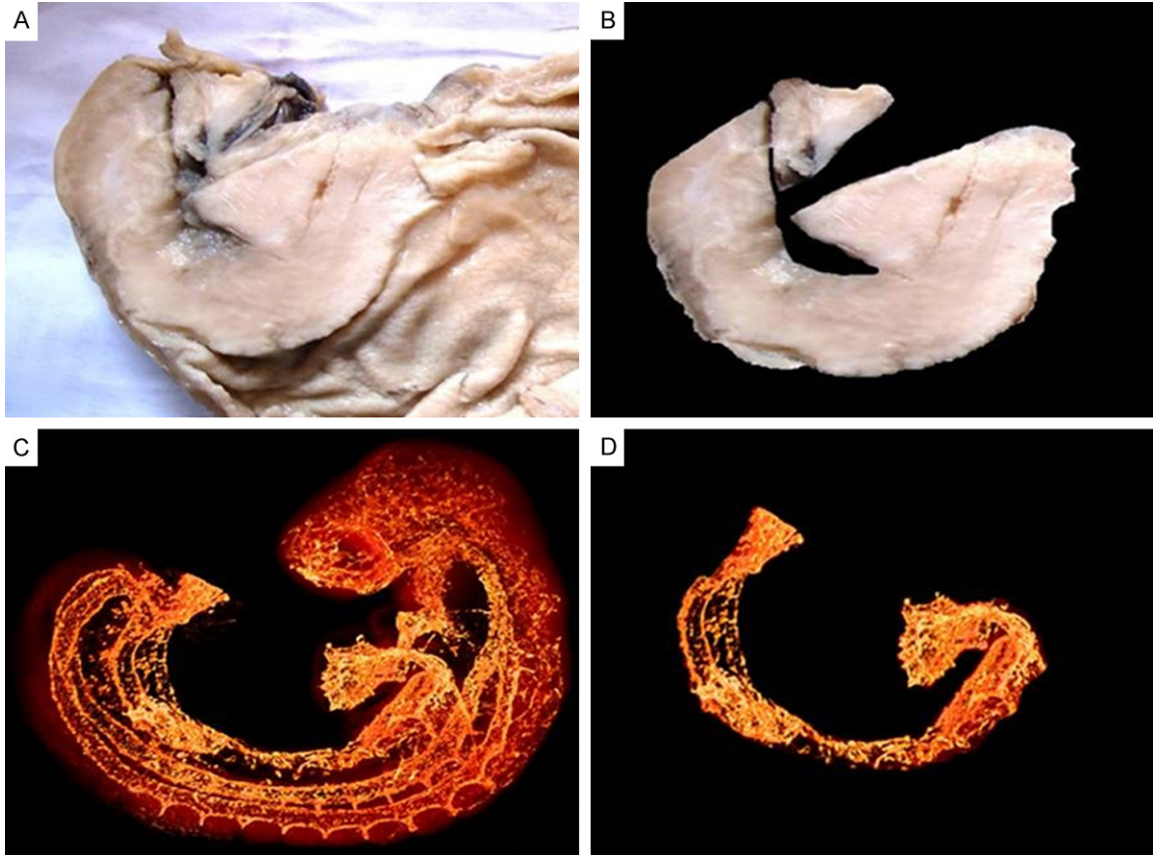
Figure 14. Comparative structural verification between self-assembly of embryoid like body in cancer hypoxic tissue with real image of three-dimensional analysis of vascular development in the mouse embryo. (A) Embryoid like structure capture in gastric cancer. (B) Detachment of image (A), (C) Real image of three-dimensional analysis of vascular development in the mouse embryo. (D) Detachment of image (C). By decomposing these images, verification, as the sequential construction of these embryos, is provided by the fractal accumulation of vascular niches of spatial order, organized by triangular chiral mirror filamentary patterns, linked by the helicoid substrate, which is a constant replication of the pattern. The fractal sum of the sequence order of the filaments results in a spontaneous self-assembly of the vascular structures with one final product: the embryo. Observe the primitive zebra stripe pattern.

These real, visible, and predictable cellular structures are located in amniotic-like cavities and show zebra-stripe segmentation characteristics. They are hyperchromatic and have high cellular 3D volumetric density because they appear in relief in relation to neighboring cells. Fully developed structures amazingly evidence cephalic-tail organization this indicates that organized cell growth polarity always exists in embryoid transformation. When these structures grow and develop, they are released from the extracellular matrix and migrate, which facilitates their detection in inflammatory effusions and especially in tumor effusions.

While all structures documented here present recognizable embryoid patterns, they are morphologically different, which shows the substantial phenotypic individuality of the originating cells and tissues. This specified complex pattern composed of several well-matched interacting parts can only be assembled by nature itself, although some form of guidance must have accounted for their origin and their presence in any other form.

Discussion

Under extreme tissue injury, human somatic cells acquire the plasticity to generate embryoid-like metamorphosis via actin cytoskeleton, a visible, reproducible, and predictable phenotype characteristic that emerges from re-expression of fetal genes, an evidence of the reverse flow of genetic information within a biological system. Malignant cells morphologically

and functionally become similar to the fetal cells of the host tissue.

Actin cytoskeleton

In accordance with our findings and literature data, in somatic cells, while the actin cytoskeleton remains intact, the cell's genetic machinery stays dormant and inactive. Stress from hypoxia or tissue injury alters this state, generating transient actin cytoskeleton degradation and facilitating the entry of the cell into mitosis [8]. This instability also leads the somatic cell to reverse its differentiation and return to its embryoid state, not only functionally but most important, as we document in this research, morphologically, and to comply with both anatomical and functional dimensions that characterize the stem cell niche as an entity in action [9].

In rat experimental models, hypertrophic stimuli induce early expression of fetal genes, including those for alpha-skeletal actin (α-SKA) and alpha-smooth muscle actin (α-SMA) [10]. Increased α-SKA expression was also observed in hearts with compensatory hypertrophy after MI [11] and in cardiac hypertrophy associated with dilated cardiomyopathy [12]. Increased α-SKA expression in the hypertrophic heart comes hand in hand with a pattern of protein distribution that is clearly different from that of the normal adult ventricular myocardium [13].

We hypothesize that this fetal actin isoform is required to achieve a high degree of myocardial contractility. This view is consistent with an earlier observation by Hewett et al. [14], who found that α-SKA mRNA is associated with increased cardiac contractility. Because α-SMA is not expressed in the healthy adult myocardium, the significance of the appearance of this isoform during cardiac hypertrophy in some animals cannot be clearly explained.

We found and documented in extreme tissue injury a particular increase in the disruption and aggregation of cytoskeletal filaments arranged in spiral-helicoid patterns that form clusters around bleeding and necrotic areas mixed with residual cellular detritus. Disruptions of the actin cytoskeleton induced by ATP depletion or ischemia have been reported in various cell types [15]. Researchers using a murine intestinal injury model demonstrated that the β-actin protein in the small intestine was cleaved and that actin filaments in the columnar epithelial cells were aggregated after a transient disruption during 30 min of ischemia, indicating that ischemia mediated the aggregation of the actin cytoskeleton, rather than its disruption. Therefore, it is evident that extreme tissue injury may alter the actin cytoskeletal network in tissues and that this action is likely mediated by the activation of fetal genes.

Additionally, researchers in this field confirm that actin-myosin filaments are able to form higher-order structures. Actin filaments are promoters of spatial order and architecture, and the reproducible shape and spatial organization of organs imply the existence of physical rules directing the assembly of complex biological structures. Organ shape and function depend on cell architecture and polarity, which are both supported by cytoskeleton networks. The formation of controlled and reproducible geometrical structures relies on the self-organization properties of actin networks and formulates the rules directing their spatial organization. Highlighting the explicit connection between the inter-filament metric properties and the geometry of cohesive assemblies of filaments is a common structural motif found in diverse contexts, ranging from biological materials such as fibrous proteins. The different actin structures governing eukaryotic cell shape and movement are determined not only by the properties of the actin filaments and associated proteins but also by geometric constraints [16-19].

The basis of the geometric and mechanical rules underlying the properties of cytoskeletal filament self-organization is contact inhibition of locomotion: when two cells collide, they each attempt to move in a different direction to avoid future collisions. As the two cells come into contact, their locomotive process is paralyzed. This is a multifaceted process that causes many cell types to repel each other upon collision. The inter-cellular actin clutch leads to a subsequent build-up in lamellar tension, triggering the development of a transient stress fiber, which orchestrates cellular repulsion. The physical coupling of the flowing actin networks during contact inhibition of locomotion acts as a mechanotransducer, allowing cells to haptically sense each other and coordinate their

behaviors [20]. In this way, actin-myosin filaments are promoters of spatially ordered architecture. GTCHC filamentous structures represent the convergence resulting from intercellular forces, dynamically orchestrated by contact inhibition of locomotion, to "avoid collisions" with each other to coordinate their behaviors.

Despite being bilaterally symmetric, most Metazoa exhibit clear, genetically determined left-right differences. In several animals, microtubule-based structures are thought to be the source of chiral information used to establish handedness. Now, two new studies in Drosophila identify a role for unconventional myosin-actin motors in this process [21].

During early *C. elegans* embryogenesis, actin plays more roles and possesses a more dynamic organization than previously described. Morphological transitions of F-actin, from meshwork to puncta, as well as asymmetric redistribution, are regulated by actin proteins. Results from the present study provide new insights into the cellular and developmental roles of the actin cytoskeleton [22].

Planarian morphogenesis has demonstrated that cytoskeletal proteins are strongly implicated in the differentiation processes that also occur in higher animals. In particular, the findings reported in planarian show that a specific actin isoform is a marker of neoblast activation and differentiation, and its modulation during morphogenetic phenomena suggests that this isoactin is necessary for regeneration [23].

Actin filaments regulate assembly during Drosophila brain metamorphosis [24], and they participate in the shape formation of mesenteric mesothelial cells of the bullfrog intercellular junctions between the mesothelial cells, which develop as the cell shape becomes polygonal during metamorphosis.

Adult wound repair differs drastically from embryonic repair. A striking feature of embryonic repair is the formation of a supracellular actin-myosin purse string at the leading edge of the wound. This purse string was first described as a mechanism for wound repair in the embryonic chick wing bud, but it has since been observed in mouse, frog, and fly embryos [25-27]. Blocking the assembly of new filamentous actin with cytochalasin D in the mouse embryo results in a failure to re-epithelialize the wound such that it remains open long after control wounds have healed.

Cell mechanics plays a role in stem cell reprogramming and differentiation. Measurements of actin stress showed that the tension in actin fibers in stem cells is higher than that in normal cells [28]. Understanding the self-assembly of actin filaments into higher-order cell structures is central to understanding cell physiology and the many linked reactions such as gene expression induced by the stress of mechanical intercellular collisions.

As we can see, in normal developmental biology, cytoskeletal filaments play an evolutionarily conserved pivotal role in morphogenesis, embryogenesis, metamorphosis and regeneration. Our observations indicate that the function of the actin cytoskeleton in emergent responses to tissue injury is reconstituted and reactivated and leads to increased gene expression, and that our body uses direct reverse flow of a genetic repair mechanism to correct the damaged bases.

Hypoxia

The ELM of somatic cells represents an adaptive response to the deprivation of oxygen, a short transitional period before the cell enters death, collapse, and tissue decomposition. ELM is also probably the last opportunity for clusters of hypoxic somatic cells to change their shape to a primitive embryogenic phenotype with one sole objective: to survive.

Hypoxia generates hypoxia-inducible factors (HIFs), transcription factors that respond to changes in the available oxygen in the cellular environment. Specifically, to decrease oxygen consumption, the HIF signaling cascade mediates the effects of hypoxia, the state of low oxygen concentration, on the cell. Hypoxia often keeps cells from differentiating. However, hypoxia promotes the formation of blood vessels and is important for the formation of a vascular system in embryos and cancer tumors. HIF signaling, when stabilized by hypoxic conditions, upregulates several fetal genes to promote survival in low-oxygen conditions. Recently, HIFs have been shown to activate specific signaling pathways, such as Notch, and the expression of transcription factors, such as Oct4, that control

stem cell self-renewal and multipotency [29-33]. Embryoid-like metamorphosis represents an adaptive mechanism to promote the survival of clusters of somatic cells under conditions that are not survivable for individual cells.

Normal mammalian embryonic development occurs in a hypoxic environment, and hypoxia is, therefore, responsible for aspects of embryonic development [34-38] (1). Later, in a hypoxic environment, the somatic cell must change its basic oxygen requirement in order to survive. The only way to achieve it is by performing an embryoid transformation.

How hypoxia promotes cellular differentiation into ectodermal neural tissue, an adaptive response to injury and cellular hypoxia, has been shown experimentally. In 1947, Holtfreter observed neural induction in explants that had undergone a sub-lethal cytolysis [39]. More recently, Lin Cheng et al. observed the generation of ectodermal neural progenitor cells using chemical cocktails and hypoxia [40]. In addition, in mice, intermittent hypoxia mobilizes bone marrow-derived very small embryonic-like stem cells and activates developmental, transcriptional programs [41]. This shows the great plasticity of somatic cells and how hypoxia is intimately related to embryogenesis and morphogenesis and is the mediator by which memory cell groups are copied from DNA templates, allowing this information to proliferate and reactivate our most primitive genes, resulting in embryoid-like transformation of injured somatic cells.

Neovascularization

Hypoxia promotes neovascularization via the formation of microvessels in solid tumors that exhibit a large series of severe structural and functional abnormalities. They are often dilated, tortuous, elongated, and saccular. There is a significant arterial-venous shunt perfusion accompanied by a chaotic vascular organization [42].

Our documentary evidence contradicts these observations, and we can say that not all is complete chaos. There are microscopic vascular niches of organization that exist in hypoxic tissues in cancer and in damaged tissues that have not previously been documented.

Filamentary vascular niches of organization represented by synchronic vasodilation and vasoconstriction functional units documented in areas of hypoxia in cancer and damaged tissues are generated by filamentary geometric attractors that are derived from intercellular collisions. This organizational niche operates and regulates the platform scaffold in which the primordial embryoid-like developmental patterning is built by fractal copies. This observation is supported by the literature: vascular niches found in other tissues control organ regeneration, and direct contact between cells within the vascular niche maintains quiescent stem cells [43, 44]. This means that spatial areas of dipole synchronism, vasoconstriction, and vasodilation are an ideal fertile microenvironment for stem cell activity. Then, the spontaneous assembly of embryoid-like structures could, in reality, be stem cell niches.

Immunostaining for neuron-specific enolase

We observed a highly selective distribution of immunostaining using the antibody against neuron-specific enolase that was related to the clusters of cells organized in an embryoid body like the phenotype assemblage in all of the examined injured tissues: skeletal muscle and pancreatic, renal, and placental tissue. The placenta consists of a fetal and a maternal component, skeletal muscle derives from mesoderm, and pancreatic and renal tissue derive from endoderm. We documented here embryoid-like structures in tissues from all of three germ layers, and all of them show positive immunostaining for a marker of neuroectoderm differentiation. This provides clear evidence that such structures may be a real injury stem cell niche as a functional entity and that they could be used in cancer treatment and regenerative medicine.

Based on the evidence, we believe it is necessary and urgent to perform a conceptual, methodological, and biological review of the meaning of cancer as we understand it today. Cancer does not produce killer masses; on the contrary, it generates and initiates emergent proliferative compartments that act in reverse takeover, generating visible and measurable products of biologic metamorphosis mediated by injured hypoxic tissues. In cancer tissues, fetal genes are reprogrammed structurally and

morphologically, as a consequence of the reverse flow of genetic information within a biological system, contributing to a metaplastic transformation with structural phenotypic replication and spontaneous self-assembly of past forms that mimic embryoid-like structures, the influence of reverse patterns on present ones. In this context, the past repeats itself, having its own laws and dynamics, and is an entity in action. Cell subpopulations in all malignant tissues have, in essence, an embryoid lineage visualized as subsets of the universal pattern of a phylogenetic tree. Our current anti-cancer arsenal probably destroys these emergent populations.

We have rich, vivid examples of the re-expression of fetal genes: The Notch signaling pathway plays an important role in adult cell-cell communication and also regulates embryonic development. Genes that are active during embryogenesis have also been identified in many types of cancer [45]. Cancer cells introduced into developing embryos can be committed to a complete reversion of their malignant phenotype [46]. Tumors share several morphological and ultra-structural features with embryonic cells, and cancer cells exposed to specific embryonic morphogenetic fields undergo significant modifications, eventually leading to a complete phenotypic reversion.

Implanted sarcoma progressed in 80% of adult rats but only in 6.4% of rat embryos, and similar data have recently been shown for chick and other kinds of embryos that are able to tame aggressive cancer cells when these are implanted [47-49].

In biology, self-assembly refers to processes in which a disordered system with preexisting components forms an organized structure or pattern because of specific local interactions among the components themselves; without external direction, the self-assembled structure must have a higher order. A direct consequence is the general tendency of self-assembled structures to be relatively free of defects, and this refers to the minimum number of units needed to make an order. Here, we are dealing with a true biological machinery of reversing system information. Recursive ontogeny recapitulates an earlier stage of development in collective stem cell memory activation, which produces a replaced copy of "neural organizer proteins" and indirectly demonstrates the existence of molecular ancestral memory that permits such regression by transmitting heritable information to a point at which most of our primitive genes are reactivated. We believe that these features clearly differentiate the ectodermal neuron-specific enolase immunopositivity embryoid-like phenotype generated by this adaptive metamorphosis of somatic cells in response to chronic and acute hypoxic injury. This phenotype may represent an active population of adult stem cells, one of the most important epigenetic phenomena for obtaining perfect and stable autologous stem cells, fully functional stem cells that come from damaged adult tissues instead of embryos or reprogramming tools.

Our findings document how under hypoxia, tissues reactivated ancestral storage memory and elaborate, high-fidelity crystalline fractal chiral structures repaired copies of the damaged substrate tissue. The resultant embryoid-like DNA template probably guides and controls the regenerative pathway mechanism in human tissues as follows: 1) Modify and reprogram the phenotype of the tumor where these structures are generated. 2) Establish a reverse primordial microscopic mold to use the collective behavior of cellular building blocks to regenerate injured tissues. 3) Convert cancer cells to a normal phenotype by developmental patterning of active patterning cues. 4) Convert cancer cells to a normal phenotype by regeneration using the organizational level and scale properties of reverse genetic guidance. 5) Globally control mitotic activity and morphogenetic movements.

Tumorigenesis mimics a self-organizing process of early embryo development. All malignant tumors produce fetal proteins, we now know from which these proteins proceed Embryoid-like metamorphosis phenomena would represent the anatomical and functional entity of the injury stem cell niche, but further studies must be carried out regarding these real, visible, and predictable adaptive responses. The sufficiently fast identification, isolation, culture, and expansion of these self-organized structures or genetically derived products could, in our opinion, be used to develop new therapeutic strategies against cancer and in regenerative medicine.

Disclosure of conflict of interest

None.

Authors' contribution

J.A.D. and M.F.M. guided the project, wrote the paper, and analyzed the results. J.A.M., M.A.B., L.S.P., L.K.S., L.C.P., and K.T.M. processed the samples.

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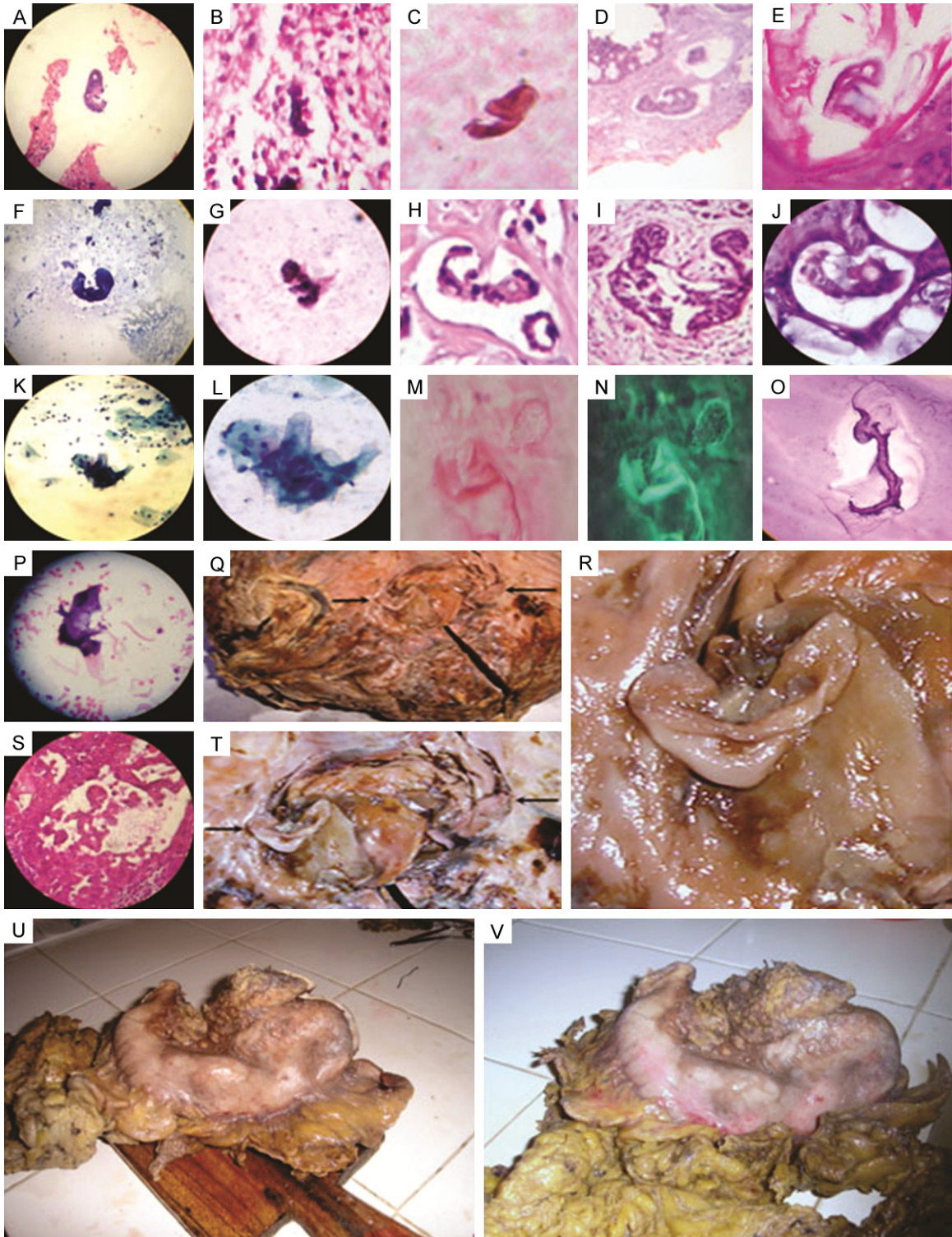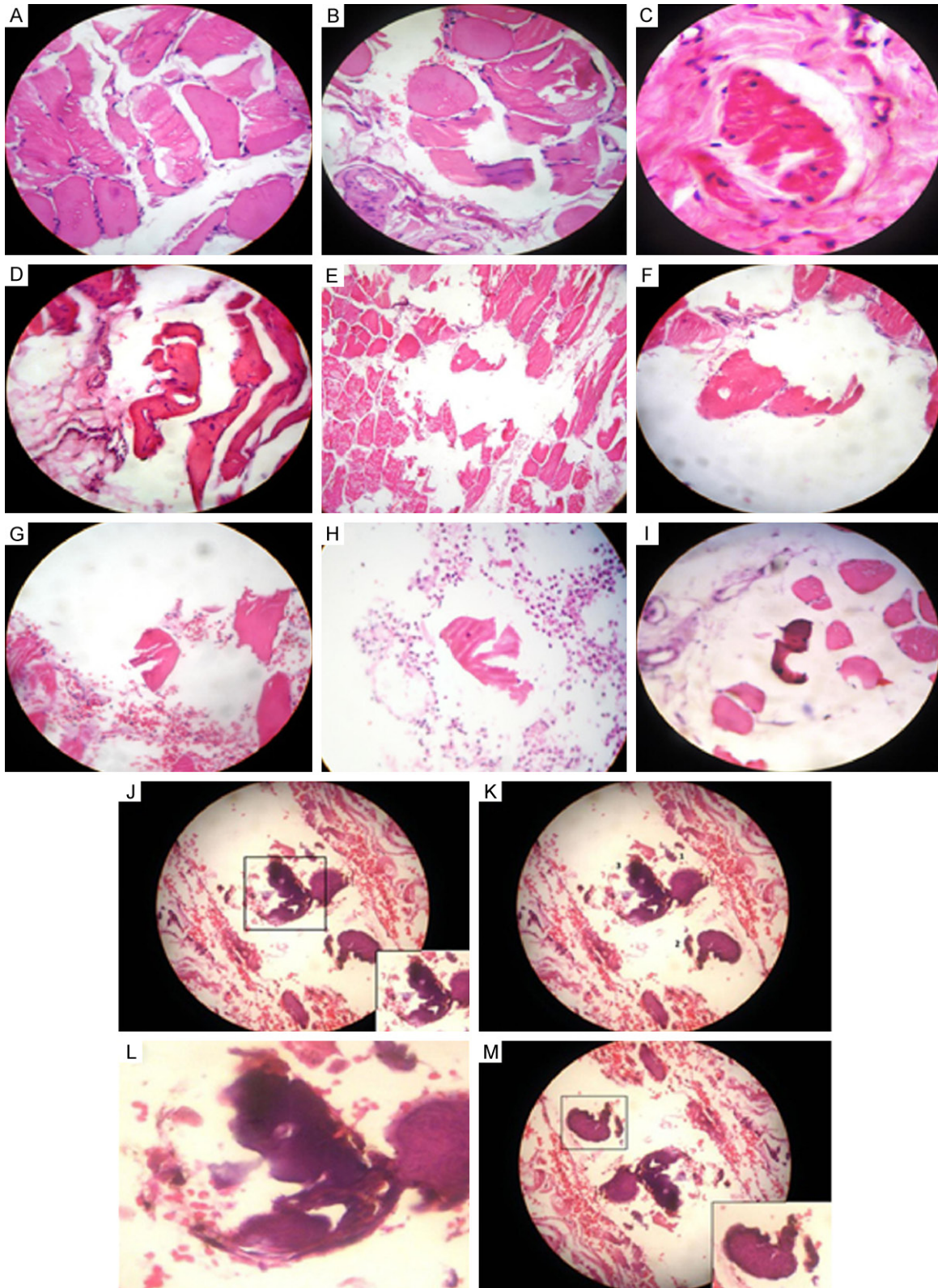
Figure S1. Photomicrographs show evidence of embryoid like metamorphosis of somatic cells in response to hypoxic injured tissues in cancer. hematoxylin and eosin staining (20×): A. Gastric area of cancer. B. Brain astrocytoma tumor. C. Undifferentiated tumor necrosis area. D. Breast adenocarcinoma. E. Malignant PIlar tumor. F. Effusion from peritoneal carcinomatosis papanicolaou staining (20×). G. Urine cytology from patient with bladder carcinoma. panicolaou staining (20×). H, I. Prostate adenocarcinoma. J. Colon adenocarcinoma. K, L. Cervical smear from patient with carcinoma in situ; papanicolaou staining (20×). M, N. Effusions from peritoneal carcinomatosis; Papanicolaou staining (20×). O. Effusion from carcinomatosis. P. Glioblastoma. Q. skin squamous cellular carcinoma. R-T. In vivo macroscopic documentation of peritoneal leiomyosarcoma, observes the embryoid structures in the surface of the tumor. U, V. In vivo macroscopic representation of diffuses gastric cancer; observe the great and minor curvature of the stomach w ith complete embryoid like metamorphosis.

Figure S2. Photomicrographs shows evidence of embryoid like metamorphosis in injured tissues from traumatic and non-traumatic amputations. hematoxylin and eosin staining (20×); (A-F) Transformation of damage skeletal muscle cells in response to hypoxia, illustrates sequential embryoid like transformation in traumatic amputation. Observe on image (A). In the center of the field, the visible hypertrophic actin cytoskeleton filaments with formation of zebra-striped pattern along anterior-posterior axis, phenomena restricted to skeletal muscle cells in transit to embryoid like metamorphosis. (G-I) Illustrates sequential embryoid like transformation of mesodermal skeletal muscle cells in response to hypoxia in diabetes vascular disease non-traumatic amputation. Observe in (H) Embryoid structure generated by hypertrophic actin-myosin filaments with formation of zebra-striped pattern along anterior-posterior axis with acute inflammatory infiltrate. (J-M) Illustrates well-defined embryoid transformation in response to hypoxia in extreme injured tissue traumatic amputation.
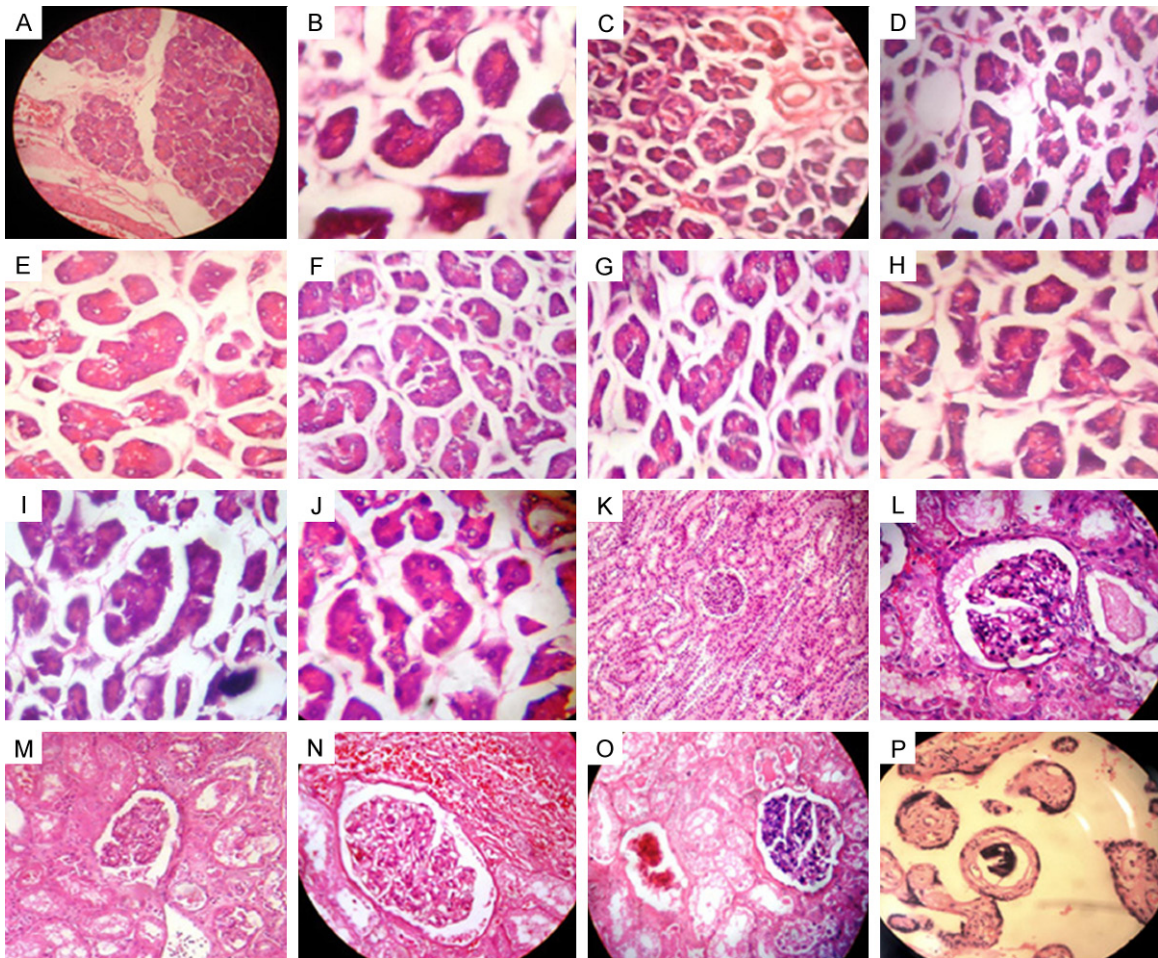

Figure S3. Photomicrographs shows evidence of embryoid like metamorphosis of pancreatic and renal glomerular cells, endodermal origin, in response to hypoxia in acute extreme injured tissues. Hematoxilin and eosin staining (20×): (A) Pancreatic normal cells control. (B-J) Embryoid transformation of pancreatic cells in response to acute hypoxic tissue damag. (K) Renal normal glomerular cells control. (L-O) Embryoid like transformation of glomerular cells in response to acute hypoxic tissue damage. Observe in (O). Embryoid like transformation adjacent to normal glomerulus. (P) Embryoid like transformation of placental cells in response to fetal hypoxia.