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Unraveling the Enigma of Organismal Death: Insights, Implications, and Unexplored Frontiers

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Significant knowledge gaps exist regarding the responses of cells, tissues, and organs to organismal death. Examining the survival mechanisms influenced by metabolism and environment, this research has the potential to transform regenerative medicine, redefine legal death, and provide insights into life's physiological limits, paralleling inquiries in embryogenesis.

organismal death; physiological limits; survival mechanisms; transformation; transitions

Introduction

A continuing enigma in the study of biological systems is what happens to multicellular organisms when their regulatory systems gradually or suddenly become disabled. In life, genetic and epigenetic networks precisely coordinate the expression of genes; however, most networks fail in organismal death (1), yet some continue to function despite loss of the "whole," as certain organs, tissues, and cells survive well, as exemplified by organ harvesting of cadavers for transplantation and by immortal HeLa and MM1S cells that are propagated by scientists for medical research. In many ways, this raises some of the same fundamental questions of multiscale biology as the inverse process, embryogenesis, in which the large-scale integrated self first arises as an emerging property from a collective of individual cells (2, 3).

Research in the field of organismal death is crucial and relevant today because our traditional understanding of organismal death and the definitions of "life" and "an organism" may be outdated. One example highlighting the need for new research is determination of the optimal time for procuring organs for transplantation from a donor. The distinction between being alive and being dead is uncertain, as new research suggests that brain circulation and cellular functions can be at least partially restored by medical interventions (4). Moreover, some organs, tissues, and cells in the body remain alive despite the lack of overall bodily function. The transition from a living body to a fully decomposed corpse has led to the new concept of the "twilight of death" (5), which is fertile ground for scientific inquiry.

Another example highlighting a curious opportunity for new research is the potential for medical interventions, such as providing nutrients, oxygen, endogenous bioelectricity, and biochemicals, to reverse the state of "dead entities" back to life or trigger a transformation into something new. Recent studies offer support for the idea of a "transformation into something new." For instance, skin cells taken from frog embryos, when given the opportunity to regain multicellularity in vitro, have been transformed into novel entities (3). These cells reassemble, utilize cilia for movement, repair damage, interact with their environment, and display spontaneous behavior while repurposing some of their original functions. They are called "xenobots" to emphasize the plasticity of the cellular collective, enabling form and function distinct from their default evolutionary end points and thus useful as a biorobotic platform. This plasticity extends beyond amphibians and embryonic stages. Recent research has demonstrated similar outcomes with adult human cells (6), indicating that even elderly components can adopt new multicellular existences when removed from the body. The evolutionary forces driving postmortem processes and xenobots' behavior are intriguing questions, possibly reflecting natural by-products of evolution not commonly observed in nature.

Overall, research in these areas challenges our conventional understanding of death, life, and the nature of organisms. It highlights the need to explore new frontiers to expand our knowledge and redefine these concepts in light of the emerging discoveries. As we delve into these intriguing subjects, numerous pertinent questions arise, such as

- As organismal death unfolds, do cells of the human body attempt postmortem survival?
- What are the underlying mechanisms of slow (prolonged) and fast (traumatic) death?

- Why are developmental genes induced upon organismal death?
- What role does "stemness" play in response to stress/organismal death?
- Do higher-order selves disappear at death?
- What is death of a multicellular organism when some of its individual cells are still alive?
- What factors contribute to the resilience and survival of certain cells and tissues after organismal death or at the twilight of death?
- Can medical interventions reverse the state of "dead entities" or trigger transformative processes leading to new forms?
- What implications do these discoveries have for regenerative medicine and the prevention of premature mortality?
- How much learned information (through life) can be kept in the living cells and tissues in the twilight of death?

By addressing these questions on the mysteries of life and death, we are poised to uncover groundbreaking insights into the very fabric of life.

The authors gathered for a 2-day workshop to exchange ideas about potential answers to these questions. This review summarizes the state of the field, showcasing the latest achievements, future directions, and relevance to biologists, medical doctors, as well as philosophers. We attempted to make each section as self-contained as possible, allowing readers to approach and understand each section independently from the others.

Long-Term Postmortem "Survivors"

Cellular Survival Signals in Postmortem Skin Tissue

Postmortem samples offer a valuable resource for investigating gene expression patterns and tissue specificity within individuals, particularly for organs and tissues that are challenging to obtain from living subjects. Understanding how death and various terminal states affect biological processes is crucial for interpreting postmortem gene expression data as a means of understanding physiological conditions.

To better understand variations in gene expression during different durations of the terminal phase of human life, 701 skin samples were collected from the Genotype-Tissue Expression (GTEx) Project (7). Skin samples were chosen because they are well suited to survive as a tissue after human death. As reported (8), the human samples with a fast death (trauma, e.g., Hardy scale of 1; Ref. 9) or a longer terminal phase (slow, e.g., Hardy scale of 4) of death appeared separated from each other according to gene expression profiles (FIGURE 1). Differentially expressed genes (DEGs, protein coding genes) were identified in skin samples based on the comparison between slow and fast death cases, suggesting that postmortem gene expression was dependent on the duration of the patient's demise. Interestingly, the skin of humans who have already undergone slow (as opposed to fast) death shows induction of developmental pathway genes (8). In such skin samples, survival pathways such as phosphatidylinositol 3-kinase (PI3K)-Akt, FoxO, hypoxia-inducible factor (HIF)-1, TNF signaling, as well as focal adhesion were induced. Upregulated DEGs were also significantly enriched in some Reactome pathways, e.g., cytokine interleukin signaling and cascade reactions involving Toll-like receptors. The protein-protein interaction (PPI) network analysis showed that these pathways are all connected by shared DEGs or the interactions among these DEGs. PI3K-Akt signaling is well known as a developmental and survival pathway that regulates cell differentiation and proliferation. More specifically, it is involved in upregulation of embryonic developmental transcription factors: Foxo1, Foxo3, ATF4, and CEBPD (8).

The skin of patients who have undergone slow death also showed induction of angiogenic pathways in the dermal endothelial cell compartment. Single-nucleus RNA-seq (snRNA-seq) data from 5,327 postmortem skin nuclei ($n = 3$, GTEx database) were analyzed. It was observed that fibroblasts demonstrated greater resilience to death of the host human. Cell-cell interaction analyses among different skin compartments using CellChat (10) revealed that in slow death subjects ~50% of the resilient fibroblasts were actively communicating with other cells (8). It was evident that gene expression in the skin samples is subject to epigenetic control.

In summary, these findings indicate that the fibroblasts are almost "unaware" that the host is gone, and they continue to operate and communicate. The big question remains, why?

Dynamics and Regulation of Postmortem Transcriptomes

The tissue samples in the previous study were collected from human donors within 24 h of cardiac cessation. This raises the question of whether the extracted transcripts changed dynamically within the postmortem period (e.g., in the 1st postmortem hour vs. the 24th hour). It is conceivable that observed differences among the samples could be attributed to variations in collection times.

To address this question, controlled experiments were conducted to monitor the postmortem abundances of transcripts in zebrafish and house mice over 48–96 h postmortem with a calibrated approach. The aim was to identify transcripts that increased in abundance with postmortem time relative to flash-frozen live controls and assess their functions.

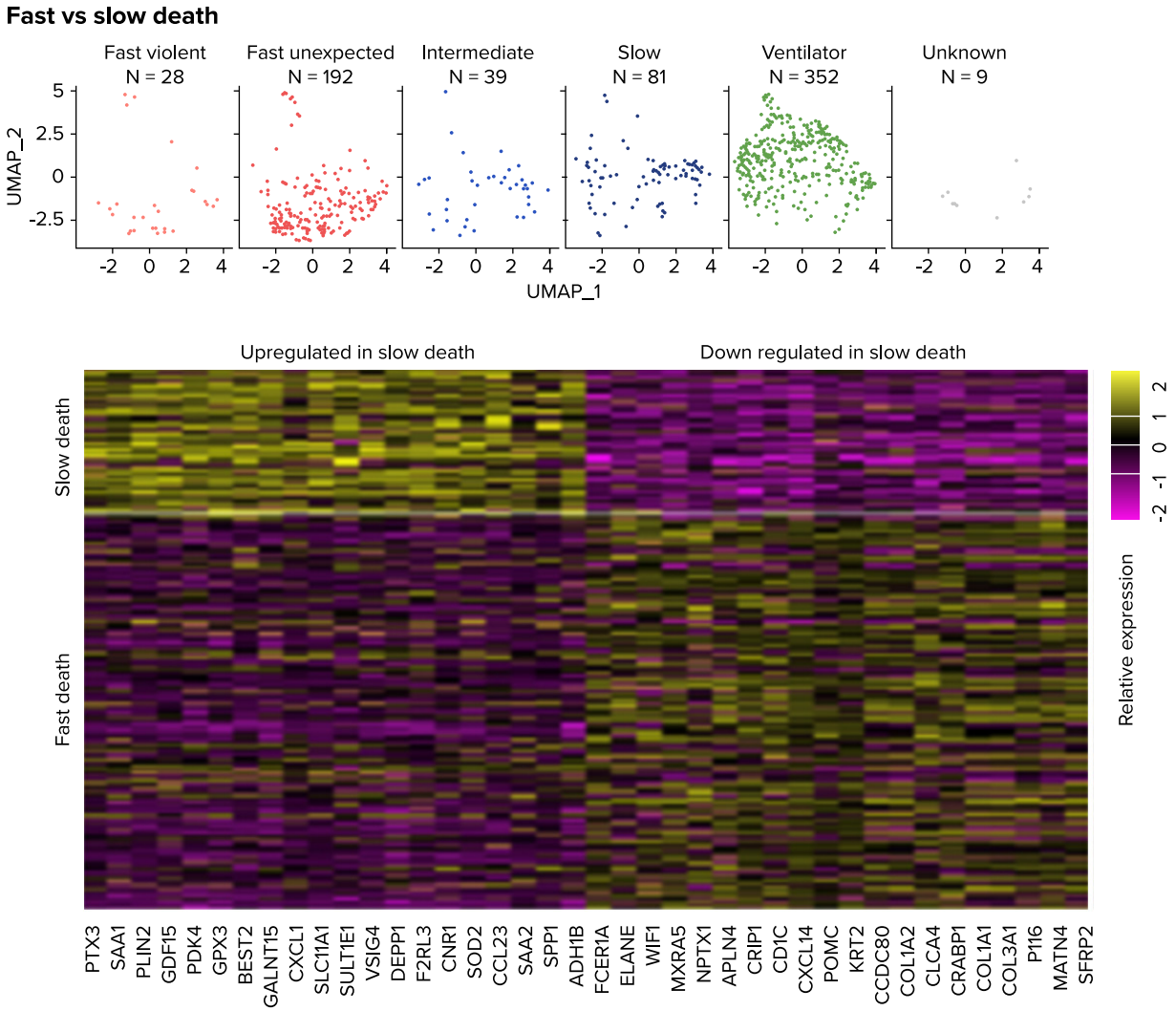


FIGURE 1. **Analysis of Genotype-Tissue Expression (GTEx) transcriptomic data in human postmortem skin**
Analysis of GTEx transcriptomic data in human postmortem skin based on type of death. *Top*: Uniform Manifold Approximation and Projection (UMAP) plot showing total human skin samples ($n = 701$). *Bottom*: heat map representing the top 20 upregulated and top 20 downregulated differentially expressed protein coding genes (DEGs) in skin cells of slow death type cases compared to fast death type cases. Gene values are log normalized. Figure adapted from Ref. 8, with permission from *BioRxiv*.

The first experiment was conducted on whole zebrafish, and the second experiment focused on the brain and liver of mice (1). FIGURE 2 shows typical increases in transcript abundances with the "Gene Meter" approach, which does not rely on usual assumptions for normalization but rather utilizes directly calibrated probes (11–13). Although only 2% of transcripts increased, ordination plots revealed nonrandom patterns of these transcripts that changed with postmortem time. These results were surprising because the researchers originally hypothesized that all transcripts should decrease in abundance with postmortem time. Gene transcripts that increased in abundance coded for stress, immunity, inflammation, apoptosis, transport, development, epigenetic regulation, and cancer. The findings were corroborated by RNA sequencing studies (14, 15) and peptide identifications (16).

Why the increase in transcript abundances? Possible reasons include *1*) thermodynamic and kinetic conditions encountered during organismal death reflecting pathways used in life; *2*) enrichment of stable nondegrading RNA in the transcription pool; and *3*) changing of cell types with postmortem time. The first reason is putatively supported by the observation that transcripts from histone modification pathways increased, which could be responsible for the unraveling of tightly wrapped nucleosomes and, in turn, the upregulation of transcription factors and RNA polymerase. Transcripts encoding nucleopore proteins are another example. The proteins allow the exchange of mRNA and other molecules between the mitochondria and the cytosol (e.g., Tpr, Tnpo1, Lrrc59) or the ion/solute protein channels (e.g., Aralar2, Slc38a4) that control intracellular ion pathways. The second reason is not supported, as an investigation into the enrichment of nondegrading RNA showed that it was not a significant factor (<5%) (1). The third reason is partially supported, as there is evidence of changing cell types, which can explain

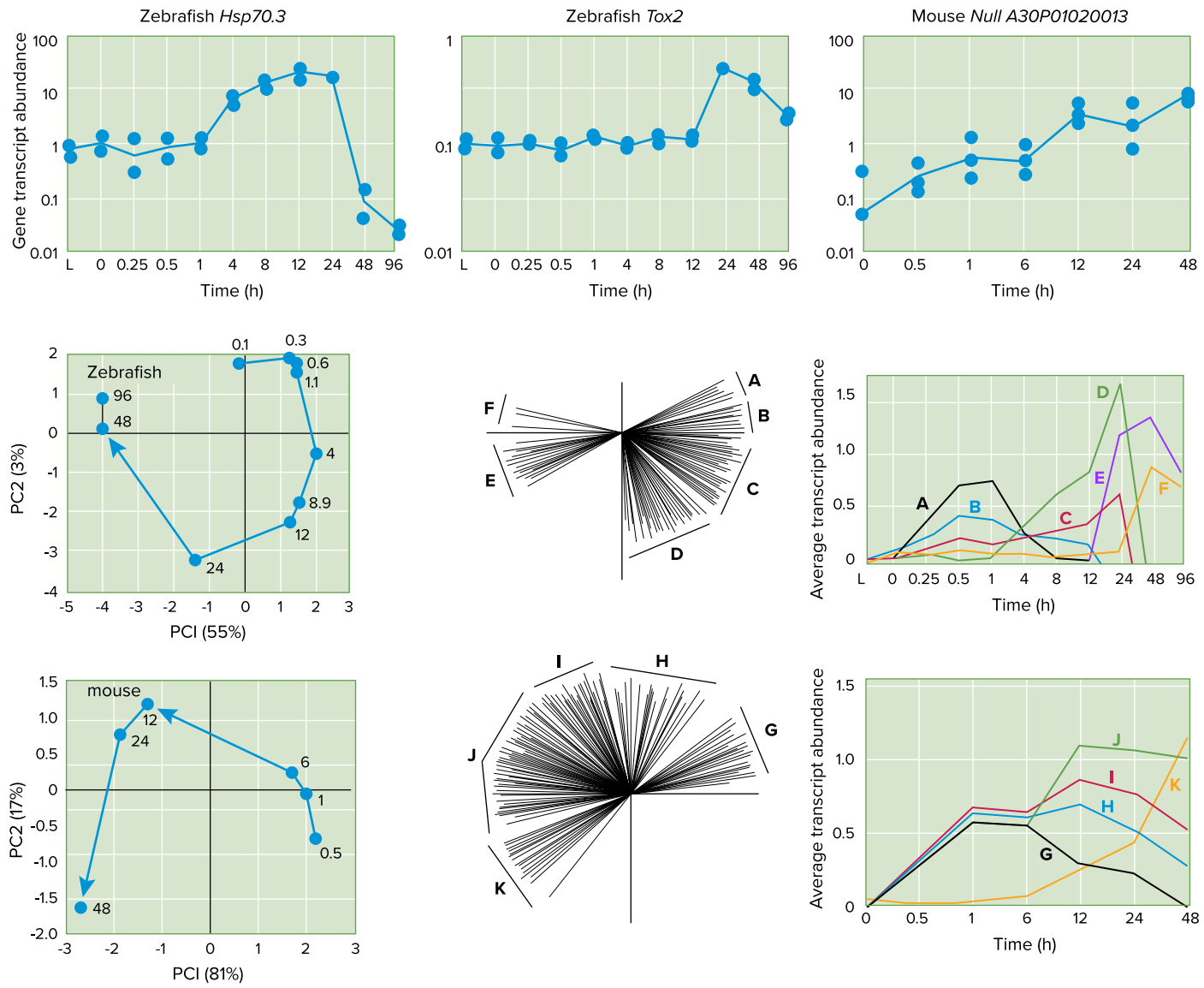


FIGURE 2. **Transcriptional profiles of representative genes**
Transcriptional profiles of representative genes (arbitrary units), ordination plots based on transcript abundances by postmortem time (h) with corresponding transcript contributions (biplots), and averaged transcript abundances by group. *Top*: transcriptional profiles of the Hsp70.3 gene (*left*), the Tox2 gene (*center*), and a nonannotated transcript "NULL" (i.e. no annotation, probe number shown) gene as a function of postmortem time (*right*). *Middle* and *bottom*: ordination plots of the zebrafish (*middle*) and mouse (*bottom*) were based on all gene transcript profiles found to have a significantly increased abundance. Gene transcripts in the biplots were arbitrarily assigned alphabetical groups based on their positions in the ordination. The average transcript abundances for each group are shown. Figure adapted from Ref. 1, per the Open Access terms of *Open Biology*.

the increased transcript abundances during postmortem time. Specifically, Dachet et al. (17) showed activation and outgrowth of microglia and astrocytes in human brains after organismal death. The increases in transcript abundances are putatively due to changes in pathways used in life and changes in cell types during postmortem time.

A follow-up study investigated whether increases in transcript abundances result from RNA binding proteins and/or noncoding RNAs by examining transcript sequence features (3- to 9-mers) putatively serving as binding sites for molecules involved in posttranscriptional regulation. They found that transcripts increasing in abundance had sequence features different from the rest of the transcriptome (18). Interestingly, some transcripts exhibited high-density sequence features, presumably acting as "molecular sponges" that sequester RNA binding proteins and microRNAs, thereby preventing regulatory interactions among the transcripts.

In summary, the demise of vertebrates is much more complicated than originally envisioned, as controlled experiments showed that thousands of transcripts significantly increased in abundance several hours/days postmortem relative to live samples. Although many transcripts were associated with survival and stress compensation, others were associated with epigenetic factors and developmental control. RNA

binding proteins and/or noncoding RNAs presumably play a role in regulating the increase in transcript abundances during organismal death. Future studies are needed to examine the underlying mechanisms regulating these genetic and epigenetic changes.

Equivalency of Cryopreserved Bone Marrow to Living Donor Sources

Although several studies have demonstrated the changes in the abundance of transcripts from postmortem organisms, pertinent questions remain: which types of cells remain viable, and for how long? This inquiry holds significance in medicine, particularly in the context of transplantation of hematopoietic cells. The relevance of this topic to our workshop lies in the persistent unmet demand for suitable unrelated donor grafts, despite the existence of numerous graft sources for allogeneic hematopoietic cell transplantation. Extracting hematopoietic progenitor cells from the vertebral bodies of deceased organ donors might present a viable alternative. Through preemptive screening, cryogenic banking, and the on-demand availability of cadaver-derived bone marrow recovered within hours, a refined manufacturing process can ensure sufficient cell dosages for transplantation (19).

An analysis of hematopoietic progenitor cells from 250 deceased donors revealed composition and quality comparable to those aspirated from healthy living donors' iliac crest (FIGURE 3) (Ref. 19). After cryopreservation, organ donor-derived hematopoietic progenitor cells remained stable, with $CD34^+$ cells maintaining high viability and function. Flow cytometry and secondary transplantation confirmed the presence of long-term engrafting $CD34^+CD38^-CD45RA^-CD90^+CD49f^+$ hematopoietic stem cells. Linear regressions indicated no significant predictive associations between donor-related characteristics and organ donor marrow cell quality or yield.

In summary, cryopreserved bone marrow from deceased organ donors contains viable cells useful for hematopoietic cell transplantation. The astonishing fact is that these cells maintain viability many hours after a donor is deceased.

Cusp of Transformative Transitions

Consciousness and Brain Activity during Cardiac Arrest

The conventional understanding of "brain death" occurring after 5–10 min of ischemia challenges the apparent viability of certain organs, tissues, and cells long after organismal death. For instance, cardiopulmonary resuscitation (CPR) has shown some success rates, with brain cells capable of surviving for up to 4 h

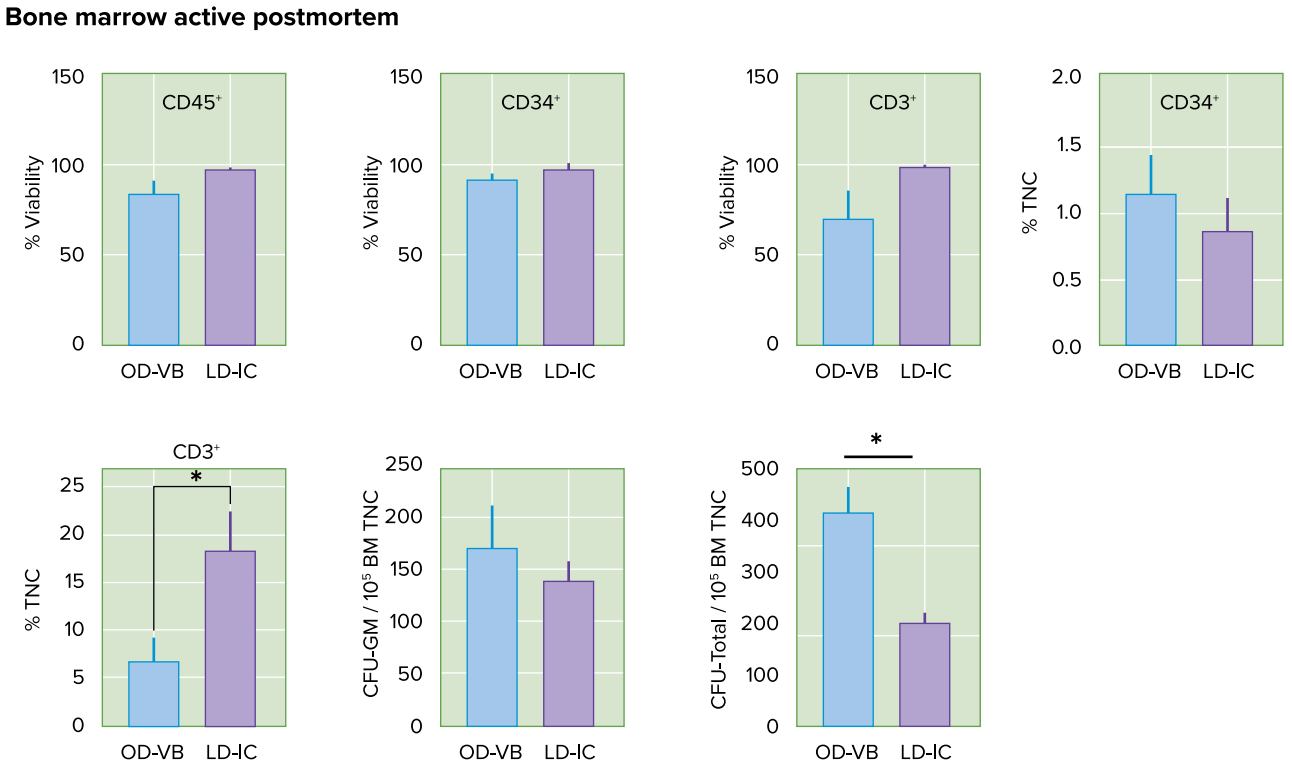


FIGURE 3. **Comparison of deceased organ donor (OD) vertebral body (VB)-derived hematopoietic progenitor cell (HPC) marrow and live donor (LD) iliac crest (IC)-aspirated bone marrow (BM)**
Comparison of deceased OD VB-derived HPC marrow and LD iliac crest-aspirated BM: viability of white blood cells (WBCs); percentage of total nucleated cells (TNCs) that are $CD3^+$ or $CD34^+$ T cells; granulocyte macrophage colony-forming unit (CFU-GM) progenitors in 10^5 whole BM cells; and numbers of total CFU progenitors in 10^5 whole BM cells. *$P < 0.05$, Welch 2-tailed *t* test. Figure adapted from Ref. 19, per the Open Access terms of *Transplant Cell Therapy*.

without oxygen (4). Furthermore, certain brain cells may retain potential for reversibility through medical interventions (20).

Parnia et al. (21, 22) have raised the question: If hypoxic brain cells are still alive, do organisms retain consciousness? The AWAreness during Resuscitation study addresses this question by examining consciousness and its underlying biomarkers during cardiopulmonary resuscitation (CPR). Specifically, they measured oxygen in the brain and electric waves by electroencephalography (EEG). Their results showed that EEG readings during CPR typically are a flat line, indicating no brain activity (21). However, bursts of brain activity in the delta, theta, and alpha waves were detected for up to 80 min (FIGURE 4*A*), and these bursts are thought to be a result of the breakdown of inhibitors that normally suppress brain activity during such circumstances.

Of 567 cardiac arrests, 9.3% of the patients survived ($n = 53$). The surviving cardiac arrest patients underwent interviews to examine recall of awareness and cognitive experiences. Approximately 52.8% ($n = 28$) completed interviews, and 39.3% ($n = 11$) of these patients reported memories/perceptions suggestive of consciousness (21). Surprisingly, patients reported common experiences during the state of clinical death, including feelings of separation from the body, a sense of going home, moving toward a destination, and a reevaluation of life, past actions, and intentions.

In summary, the traditional criterion for determining organismal death might be too stringent, as evidenced by observations of brain cells surviving hypoxic conditions for extended periods, potentially challenging established norms. Future research, focusing on interventions to revive hypoxic cells including neuronal cells, could offer opportunities to prolong life. Additionally, reported near-death experiences of surviving patients suggest the presence of consciousness, implying continued activity and functionality of some hypoxic brain cells.

Human Brain Tissue and Cells Preparing for Death

In addition to traumatic death versus a longer-term phase of death, another factor potentially influencing the postmortem survival of organs, tissues, and cells is the presence of agonal factors such as surgery, fever, and infection. How exactly do agonal factors impact gene expression upon organismal death?

To answer this question, postmortem transcriptome data from brain tissue and cells were analyzed from the Religious Orders Study and Memory and Aging Project (ROSMAP). The ROSMAP provided detailed terminal data, including information on surgery, fever, infection, unconsciousness, difficulty in breathing, and artificial ventilation for each donor 3 days before death (23).

Differential gene expression (DEG) analysis of 262 samples showed that only fever, infection, and unconsciousness were significantly associated with various gene expressions, whereas fever was positively correlated with infection and unconsciousness (FIGURE 4*B*). Coexpression network analyses further highlighted changes in microglia, endothelial cells, and neurons associated with fever and infection. These factors can impact the immune response, which, in turn, may affect neuronal activity. Specifically in fever, the brain becomes inflamed, which may cause microglia to be highly activated for immune defense. The interplay between agonal factors and the immune response highlights the complex relationship between immune function and neuronal processes in the postmortem brain.

In summary, the analyses revealed that fever and infection were contributing to gene expression changes in postmortem brains and emphasize the necessity of study designs that document and account for agonal factors. Future research could further elucidate the molecular mechanisms underlying these associations, specifically investigating how fever and infection influence gene expression in microglia and neurons. This may provide insights into the interplay between agonal factors and immune responses in the postmortem brain.

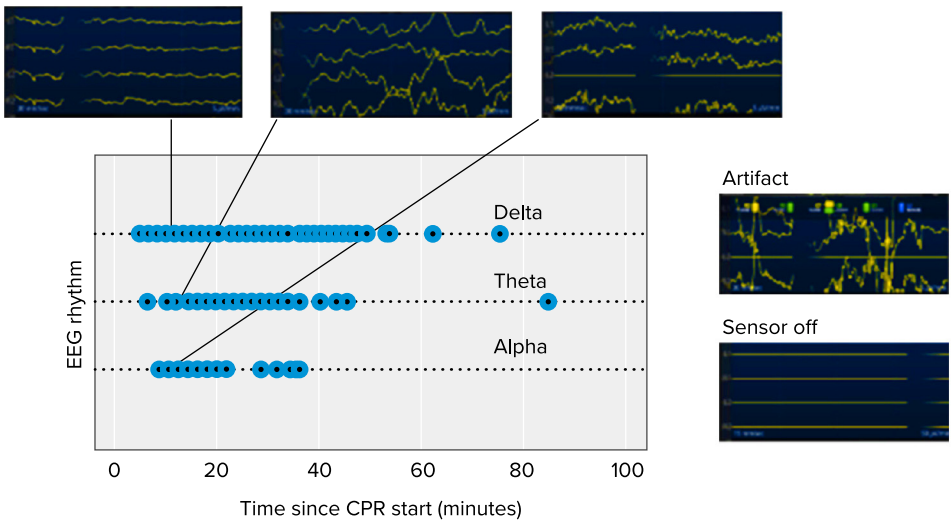
How Selves Arise, Change, and Dissolve

To understand the life and death of a complex organism, it is essential to know how coherent, high-level selves first emerge from the activity of individual cells. The Levin laboratory approached this issue by dismantling a frog embryo (at which point the original organism can be said to be dead, though the cells are alive), dissociating the epidermal cells, and providing them with the opportunity to regain multicellularity in vitro (24). Several outcomes were possible: they could spread out to form a monolayer, they could die, or they could self-organize into different types of living forms. They hypothesized that when liberated from the rest of the animal the cells could self-organize into new entities distinct from the characteristics observed in the wild-type organism, despite their unedited genome (3). Xenobots were used as a model to investigate what happens when a higher-level entity dies but its cells are still alive: what do they do? In this aquatic model, cells, unlike in mammals, continue to live, offering insights into the conatus (tendency to continue to exist) after an organism's death (3, 25).

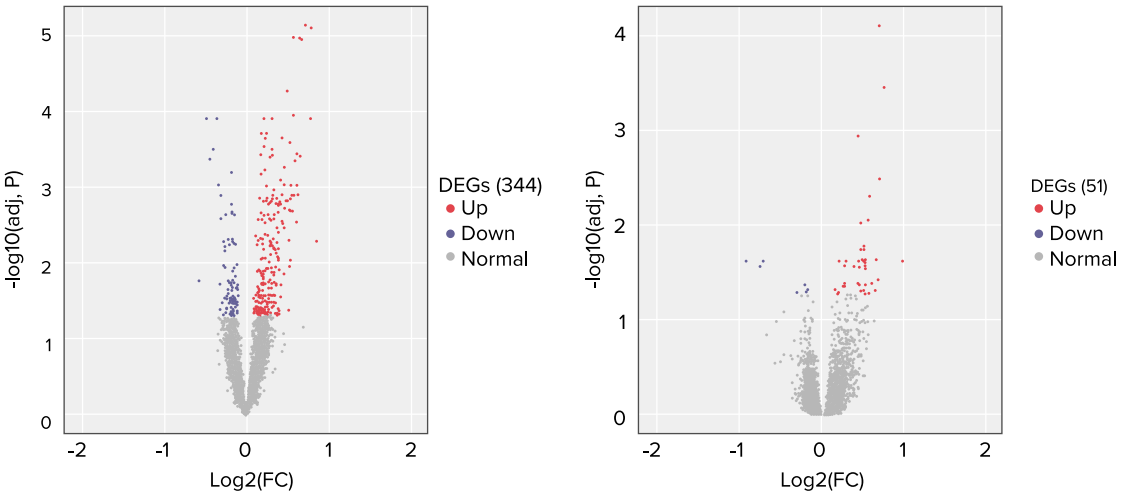
They discovered that the cells reassembled themselves to become new entities that moved through water via the action of cilia (FIGURE 4*C*). The cilia, normally used to redistribute mucus, were used to propel the organism.

They also found that the xenobots were able to repair damage, interact with their environment, and

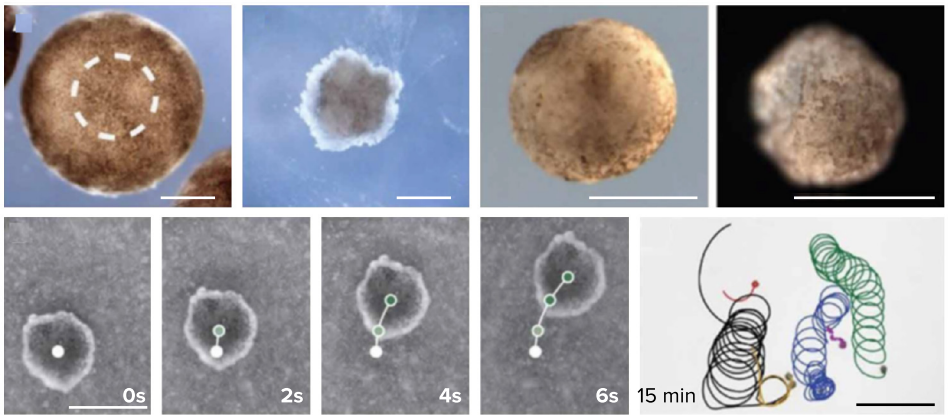
A Postmortem brain activity



B Agonal factors affect expression



C Xenobots



display spontaneous behavior. This research is just scratching the surface of what our body cells can do when extracted from their context and given the opportunity to reboot their multicellularity. Being unable to reproduce in the normal froglike fashion, they found a unique novel method, kinematic replication, in which they form novel xenobots out of loose cells they find in the environment. They have termed them "xenobots" to emphasize the fact that by uncovering and learning to control novel competencies that are not obvious from their wild-type form, we can exploit them as a bio-robotics platform (25). Crucially, human cells can do something very similar: they not only form anthrobots after their removal from an adult patient body but are able to actually heal defects in peripheral innervation (6), an entirely novel and previously unknown competency of human tracheal cells. The Levin laboratory is currently testing gene expression in xenobots and anthrobots (compared to normal embryos) and their ability to form and retain memories.

Given these findings, it is possible that the active gene expression seen after "death" may be due to the fact that individual cells are quite ready to adopt a new life in a new context, if provided with the opportunity. For amphibian and fish cells, this can be quite natural, whereas mammalian cells would require the help of a bioengineer to shepherd them to a new life after death in a nonaquatic environment.

How do individual cells coordinate activities toward reliable anatomical and functional outcomes? Data over the last two decades from the Levin laboratory and other groups show that bioelectrical signaling via transmembrane resting potentials produced by ion channels and protein pumps can help guide cells toward achieving specific goals, by encoding large-scale anatomical prepatterns (reviewed in Ref. 3). Proliferation, differentiation, migration, and apoptosis are examples of cell-level behaviors guided by bioelectrical signals, but even more important is the fact that these bioelectric states across large cell groups encode anatomical-level information such as location of whole organs and orientation of body axis. For example, by manipulating gap junction-mediated networks in planaria, it was possible to create a stable two-headed planarian that retains its two-headedness even after being cut in half and regenerating, despite its wild-time genome (26).

In summary, research in the Levin laboratory focuses on the emergence of higher selves from individual cells, whether in naturally evolved embryos or in synthetic constructs such as biobots. In both cases, biophysical modalities such as bioelectric networks (but also biochemical and biomechanical cues) serve as a kind of "cognitive glue" that binds individual competent subunits into emergent larger-scale selves (27). The potential future for regenerative medicine includes laying down proregenerative molecules, clearing plaque from arteries, healing spinal cord or retinal damage, and dealing with cancer cells or bacteria in the intestinal tract.

Mechanisms for Survival

Survival of Cells

A consistent finding highlighted in this workshop is the varying viability observed in the survival of organs, tissues, and cells after organismal death, indicating the involvement of multiple underlying mechanisms. For example, the transplantation of metabolically active islets from donors to recipients for type 1 diabetes mellitus treatment reveals several influencing factors such as isolation time, donor age/health, tissue type, storage conditions, and donor species (28). Despite the confirmed survival of cells after removal, achieving successful culture remains challenging. Autoimmune processes targeting transplanted islet cells further compound this challenge, complicating therapy because of unclear mechanisms of cell survival, likely associated with basal metabolic activities and degradation protection mechanisms. Metabolically active cells, with their high energy cost, apparently present greater difficulties in culture compared to cells with lower energy requirements.

The Joslin Medalist study examined patients who have relied on insulin therapy for $>$50 yr to understand the factors contributing to their long-term survival, identify protective factors against complications like blindness, heart, and kidney diseases, and explore how patients maintain insulin production despite autoimmune attacks. Approximately 600 patients have donated their organs postmortem, enabling investigations into cellular survival and cultivability. Skin fibroblasts were found to be culturable in 91% of cases, whereas cells from the aorta, smooth

FIGURE 4. **Different categories of normal/near-normal electroencephalography activity during cardiac arrest**
A: different categories of normal/near-normal electroencephalography (EEG) activity during cardiac arrest. CPR, cardiopulmonary resuscitation. Adapted from Ref. 21, with permission from *Resuscitation*. *B*: differentially expressed genes (DEGs) associated with the agonal factors of fever (*left*) and infection (*right*). In total, 344 significant DEGs were associated with agonal fever, and 51 significant DEGs were associated with infection. FC, fold change. Adapted from Ref. 23, with permission from *Frontiers in Neuroscience*. *C*: making of xenobots. *Top*, *left* to *right*: *1*) Tissue is harvested from the animal hemisphere of a stage 9 *Xenopus laevis* embryo. *2*) Explants are then moved to a solution and inverted for healing. *3*) After 30 min, explants heal into a spherical ball of ectoderm. *4*) Four days after formation, xenobots differentiate into irregular epidermis and are selected for experimentation. *Bottom*, *left* to *right*: time-lapse imaging reveals that xenobots are mobile when observed in an aqueous environment, with rotational biases observed when tracked for longer periods. Scale bar = 500 μm. Adapted from Ref. 24, with permission from *Science Robotics*.

muscles, retina, endothelia, and mesangia showed limited or no success in culturing. In other studies, cells from bovine eyes and aorta were successfully cultured (29), indicating that cultivability might be dependent on species origin. Additionally, RNA degradation was observed in retina, pancreas, and renal glomeruli, whereas protein degradation was significant in the pancreas but not in the aorta, retina, renal cortex, and heart.

Key observations are that *1*) the success of culturing cells and obtaining nucleic acids and proteins is highly dependent on the tissue source, including its location and species; *2*) cultured cells obtained from isolated tissues retain the disease characteristics of the donor, either as primary cells or redifferentiated from induced pluripotent stem cells (30), indicating the presence of a metabolic memory (31); *3*) when exposed to stress factors, cells transmit knowledge to progenies through non-DNA changes (30); and *4*) some cells exhibit remarkable resilience, remaining protected from disintegration even under severe hypoxia and low-nutrient conditions (32).

To understand the cell survival mechanisms, skin fibroblasts from Medalist patients were compared with controls (33). Skin fibroblasts were studied because abnormal fibroblast function underlies poor wound healing in patients with diabetes. Moreover, Medalist fibroblasts demonstrated a reduced migration response to insulin, lower VEGF expression, and less phosphorylated AKT. Medalist fibroblasts were also functionally less effective at wound closure in nude mice. The presumed reason for the poor wound healing was the presence of protein kinase C (PKC)δ (34). The results indicate that persistent PKCδ elevation in fibroblasts from diabetic patients inhibits insulin signaling and function to impair wound healing and suggest PKCδ inhibition as a potential therapy to enhance wound healing in diabetic patients.

In summary, although there are many factors affecting the ability to culture cells, some cells are more robust than others and this could be due to cellular basal metabolic activities and cellular mechanisms that protect against degradation. Metabolically active cells (i.e., high energy cost of living) are more difficult to grow and culture than cells having low energy cost of living. Identification of protective mechanisms to avoid cellular disintegration and improve survivability after cessation of nutrients will provide a significant impact on therapeutic approaches for chronic diseases such as diabetes.

Human Postmortem Microbiome

Organismal death does not happen instantly, and human organs decompose at different rates and in different ways (35). For example, human reproductive organs (prostate and uterus) are the last internal organs to decay during putrefaction. The Javan laboratory investigated the abundance and proliferation of specific putrefactive microorganisms in reproductive organs. Thirty-four reproductive organs (21 prostate glands and 13 uteri) were collected from criminal casework cadavers during the autopsy. Examination of the relative abundances of bacterial families showed that Clostridiaceae were predominant in the prostate samples (36). Conversely, bacteria in the Enterobacteriaceae and Lactobacillaceae families dominated uterus samples. Overall, there was a substantially lower abundance of the Clostridiaceae family, which contrasts to a previous study (37) where the abundance was higher in faster-decaying internal organs such as the liver and spleen (38, 39).

In summary, these studies address the paucity of information on the role microorganisms play in decomposition of organisms (40). An interesting question to ponder is what mechanisms attract various distinct microbial communities into the organs after organismal death? It is plausible that microbial processes may have evolved as a means of optimizing resource utilization, facilitating decomposition, or influencing ecological interactions. Further exploration of this question could provide valuable insights into the significance of the postmortem process.

Conclusions

The workshop was unable to address all the questions posed in the INTRODUCTION, but we did manage to tackle some of them. One significant finding is the remarkable ability of certain organs, tissues, and cells to continue functioning long after the death of a multicellular organism. However, the precise mechanisms that determine why some of these entities remain viable while others do not remain poorly understood. It is conceivable that an entity's microenvironment, metabolic activities, preservation mechanisms, and survival pathways influence the response to organismal death.

The workshop highlighted the potential importance of various dependencies in this context, including

- The manner in which the organism died (prolonged or traumatic)
- The state of the organism before death (e.g., infection, fever, or not)
- The type of entity (body, organ, tissue, cell)
- The energy requirements, such as whether the entity has a low or high cost of living
- The duration of time that the organism has been deceased
- The age, health, sex, and species of the deceased organism

It is evident that further research is imperative to unravel the complex web of dependencies and their

impacts on the underlying mechanisms governing the viability of entities such as organs, tissues, and cells.

One unresolved question is how these entities communicate across interfaces after organismal death. Research by the Levin laboratory has demonstrated that unique traits, exemplified by xenobots, can emerge from alterations in cellular communication across multiple interfaces via endogenous bioelectricity and biochemical signals. This raises the question of how organs, tissues, and cells communicate after "traumatic/fast" organismal death, such as decapitation. Unlike planarians, vertebrates cannot regenerate new heads or other body parts upon death, prompting inquiries into the reasons behind this limitation. Furthermore, it prompts speculation about the possibility of medical interventions enhancing endogenous bioelectricity and biochemical signaling in the future to generate new body parts. ■

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