

The postmortem microbiome and gene expression in vertebrates

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In life, most internal organs of a healthy adult vertebrate are essentially ‘microbial-cell-free’ – but in death, microorganisms invade and proliferate these internal organs and genes are expressed by the host presumably in response. We recently investigated how these two interconnected processes change with postmortem time and examined the feasibility of using this information to determine the postmortem interval, i.e. the elapsed-time-since-death. Although more research is needed, our findings suggest one of them has a significant potential to determine the length of time from the person dying until discovery of the body.

Before a human is born, it is essentially ‘microbial-cell-free’. But once the baby exits from the uterus, consumes food and interacts with parents/siblings and the environment, he or she becomes inoculated with microorganisms that live and thrive in the body. Exceptions to the rule are some internal organs, such as the kidneys, liver, spleen and brain. Apparently, protective barriers and the immune system prevent these organs from being colonized by microorganisms in life.

Given that there is a massive increase in microbial abundance when an animal dies, we wondered what happens to these ‘microbial-cell-free’ organs in organismal death? Do microorganisms invade and proliferate in these organs? Do differences in the biochemistry of the organs affect microbial

colonization? Would bile-tolerant microbial species, for example, flourish in the liver and species adapted to iron-rich environments proliferate in the spleen? What is the response of the host in terms of gene expression? At the time we started this work, there were no answers to these questions and that motivated us to investigate the ‘biology of death’ as it is apparently an open field for scientific discovery.

On a practical level, forensic scientists need tools to establish the elapsed time since a person has died and the postmortem investigation begins. The elapsed-time-of-death is more commonly referred to as the postmortem interval or PMI. While there are many biological, chemical and physical indicators that could be used to determine the PMI – most are not accurate. We were interested in ascertaining

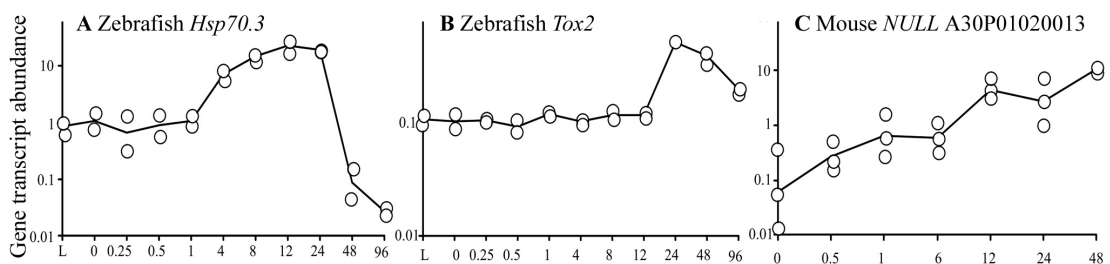


Figure 1. Transcriptional profiles of three genes, two from the zebrafish and one from the mouse. L, live flash-frozen; an open circle represents the datum point of the mRNA transcript from two zebrafish or one individual mouse.

if the following would be good predictors of PMI: presence/absence or abundance of certain bacteria in the internal organs (since microorganisms are not directly exposed to the environment) and the expression of host response genes in internal organs or whole animals.

Conventional wisdom

When an adult human dies, e.g. a massive heart attack or a gunshot wound, there is a sequential chain of events that takes place that is well known to forensic scientists: (i) cells in the body become hypoxic because the heart stops and blood circulation ceases, (ii) hypoxia triggers the release of intracellular factors that causes the organized degradation of cellular organelles by proteolytic and autolytic enzymes, (iii) vesicles, formed by these enzymes, are released into the tissue fluid where they are phagocytized, (iv) membranes of human cells are lysed by autolytic enzymes resulting in the release of cellular constituents (carbohydrates, amino acids, lipids, minerals and water) into the tissue fluids and (v) there is a massive increase in

microbial abundance because necrotic human cells release their nutrients to the surrounding tissue, and microorganisms metabolize the cellular constituents.

Postmortem microbiome

In terms of time, in the first few hours after organismal death, microorganisms cannot metabolize cellular constituents in the tissue fluid because they are suppressed by the immune system. After 24 hours, however, microbial cells overwhelm the immune system and proliferate. The microbial cells presumably enter the internal organs through the gut and respiratory systems and spread through the arterial/venous, lymphatic and mucus system in a time/temperature-dependent manner. Alternatively, a protrusion into the body or a breakage of the skin might introduce microorganisms in the blood and/or oxygen into the body. We assume that microbial community composition and abundance in the blood and organs will change with time/temperature because bacteria have different growth optima; therefore, some bacteria will utilize cellular fluids more rapidly than others.



We surveyed the postmortem microbiome of the brain, heart, spleen and blood of 11 cadavers to establish the earliest detection of microorganisms and to ascertain if the microbial composition varied by organ and/or cadaver. This was accomplished by extracting DNA, amplifying the 16S rRNA genes using PCR (polymerase chain reaction) and sequencing the amplicons using next-generation sequencing (NGS). 16S rRNA sequencing is commonly used to identify different species of bacteria due to its variability as a gene and its presence across a wide range of species.

The earliest detection of microorganisms in a liver was 20 hours postmortem and the earliest detection in other sampled organ tissues (i.e. the brain, heart, spleen and blood) was 58 hours postmortem. A putative reason microorganisms are first detected in the liver is because it receives nutrient-rich blood from the hepatic portal vein and artery and its location is in close proximity to other organs that have high microbial content (i.e. gastrointestinal tract). Despite differences in microbial detection times between organs, we found no predictable pattern of microbial distribution by organ. In fact, there was more variation between individual cadavers than there was between organs within a single cadaver.

A major drawback of our postmortem microbiome study was the method used to determine microbial community composition. Specifically, NGS was sub-optimal because the reported microbial abundances were neither accurate nor quantitative. As we showed in a recent comparative study, about 45% of the microbial species in biological samples detected by Gene Meter technology (i.e. calibrated DNA microarrays) were not detected by conventional NGS. Data mining of the Gene Meter and NGS sequencing data revealed that filter processing of the sequencing reads was the source of this problem. In addition, comparisons of NGS reads of unamplified DNA versus PCR amplified 16S rRNA genes of the same biological samples were not correlated, indicating bias in read counts presumably due to PCR amplification. Taken together, we concluded that determining the PMI using microorganisms in internal organs by NGS might not be as useful as initially envisioned and we sought other ways to explore the 'biology of death'.

Postmortem gene expression

The reason we investigated gene expression is because there is ample evidence that it takes place long after organismal death. Take, for example, Giovanni Aldini's infamous experiment where he stimulated a cadaver with an electrical current to report that 'the jaw began to quiver, and the adjoining muscles horribly contorted, and one eye actually opened'.

Hence, the body of the cadaver appeared to be 'alive' despite having no blood and a severed spinal cord. Genes have to be expressed for muscle movement; the critical question here is: how long does gene expression occur after organismal death?

Recall that messenger RNAs (mRNAs) are transcribed from genes in response to physiological demands of the cell. The 'turning on' of the genes and the production of mRNAs tells us about the inner workings of genetic regulatory networks. In death, we would expect the upregulation of response genes associated with hypoxia and stress because the heart is no longer supplying the cells with oxygen. We would also expect upregulation of immunity genes that keep microorganisms in check. We focused on the abundance of mRNA transcripts using whole zebrafish and the brains and livers of mice because transcript abundances could be precisely determined using Gene Meter technology. We constructed a fine-grain time series of postmortem transcriptome data for these animals and identified gene transcripts that significantly increased in abundance relative to live controls.

Not surprisingly, most gene transcripts of the zebrafish and mouse decreased in abundance within the first hour after death and continued to decrease for the postmortem times measured from 48 to 96 hours. The decrease in transcript abundances presumably reflects degradation and/or the downregulation of genes. Yet, about 1% of the gene transcripts (548 in the zebrafish and 515 in the mouse) significantly increased in abundance (relative to controls) from 48 to 96 hours postmortem. It should be noted that the diversity of gene transcripts (with increased abundance) declined from 24 hours postmortem in both animals, perhaps indicating that a natural threshold had been reached.

Variability in typical transcript profiles of the 1% of increased gene transcripts are shown in Figure 1. For the zebrafish, each datum point represents the mRNA from two individuals, and for the mouse, each datum point represents one individual. The transcript abundance of the Hsp70.3 gene increased after 1 hour postmortem to reach a maximum at 12 hours; the Tox2 gene increased after 12 hours postmortem to reach a maximum at 24 hours; and the NULL (non-annotated) transcript abundance consistently increased with postmortem time. The figure shows that replicated samples of the same gene transcript taken at the same postmortem time were highly similar, indicating high reproducibility of the sample replicates in their response to organismal death.

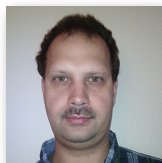
Our research showed that postmortem transcripts could be used to determine the PMI. This was accomplished by modelling parameters of the gene transcript abundances to the postmortem time, and

calculating the fit and slope of predicted versus actual postmortem times. The top predictive sets of gene transcription profiles for an animal were identified by repeating this design several thousand to a million times. Hence, by knowing the transcript abundances of a set of genes, we were able to precisely and accurately determine the PMI for a specific animal. In theory, we captured the 'genetic selfie' of a corpse (i.e. the transcript abundances of a set of genes) and used this information to determine how long a corpse has been dead. A practical outcome of our research is a validated experimental design that can now be adapted by forensic scientists to predict the PMI of cadavers.

A path forward

To date, no study has investigated the interactions between the postmortem microbiome and gene expression of the host with postmortem time. Although we investigated them separately, much more information could be attained by understanding how these two interconnected processes change with postmortem time. We emphasize this point because in life, the interactions between animals and microorganisms are not specialized occurrences but rather fundamentally important aspects of animal

biology from development to systems ecology. The question we ask is: if microorganisms play such important roles in life, why wouldn't they play important roles in organismal death? Future research in this area is needed to reveal the grey areas in the existence of microorganisms and vertebrates – in both life and death. ■



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Further reading

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