Postmortem vs. neoplastic gene expression: Clues to cancer development and therapy

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ABSTRACT

Organismal death does not immediately end gene expression. Studies of postmortem gene expression in zebrafish and mice and in the myocardium, liver, prostate, pericardial fluid, and blood of human cadavers have identified genes whose expression is increased after organismal death. Cancer can be considered a form of “un-death” since excessively proliferating cells are typically unusually resistant to apoptosis (programmed cell death), and are subject to strong selective pressure for “uncontrolled life.” The changes in gene expression observed in organismal death, particularly in mammals (mice and humans), can be compared to that observed in human neoplasia, and the comparison of these expression patterns can inform us about human cancer. Here we present a hypothesis based on the following three tenets: (a) there will be distinct and opposing patterns of gene expression between the postmortem state and cancer with respect to key physiological outputs such as growth, apoptosis, invasion, and prognosis; (b) cancer cells considered more aggressive (e.g., derived from a metastasis and/or resistant to agents that suppress growth or induce apoptosis) will exhibit expression of relevant genes more unlike that of the postmortem condition while less aggressive neoplastic cells will exhibit gene expression more similar to the postmortem condition; and (c) targeting gene expression in cancer to produce a more postmortem-like pattern will promote less tumorigenic and less aggressive cell phenotypes. To evaluate components (a) and (b) of our hypothesis, we focus on previously published gene expression data from colorectal cancer (CRC) and colonic adenoma cells and compare that to postmortem expression data. This preliminary analysis in general supports our hypothesis, with more aggressive neoplastic cell types exhibiting gene expression patterns most unlike that found in the postmortem condition; this suggests that cancer and the postmortem condition represent opposing ends of a gene expression spectrum in the balance between life and death. Subsequently, we discuss the possibilities for further testing of the hypothesis, particularly for part (c), and we also discuss the possible implications of the hypothesis for cancer therapeutics.

Introduction

Are there tissue-specific patterns of postmortem gene expression that can be contrasted to that of normal and cancer cells? Does postmortem gene expression, associated with death, contrast with that of neoplasia, associated with “uncontrolled life” (enhanced proliferative potential, inhibited apoptosis, and immortalization)?

Studies on zebrafish (Danio rerio) and mouse (Mus musculus) have identified 1063 genes with upregulated expression after organismal death; in some cases, altered expression occurred up to 48 hr postmortem [1]. Identified genes fell into several categories, including stress, immunity, inflammation, apoptosis, transport, development, epigenetic regulation, and cancer [1].

A small number of genes exhibit upregulated expression postmortem (at 12 h) in the myocardium, pericardial fluid, and blood of human cadavers [2]. In addition, studies on postmortem gene expression in the human liver and prostate have identified a larger number of differentially expressed genes [3,4]. In the liver, there was a general trend for repressed expression of anti-apoptotic genes (e.g., BIRC5, BCL2, BCL2L2, BCL10, etc.) and enhanced expression of pro-apoptotic genes such as caspases (e.g., CASP, CASP4, and CASP9) [3]. However, this was not a simple uncontrolled pattern of dying cells increasing apoptosis as the anti-apoptotic gene XIAP also demonstrated significant upregulation of postmortem expression, and other studies have shown ongoing control of tissue-specific gene expression in postmortem tissues [5]. The prostate is relatively decay-resistant and is among the last internal organs to decompose after death; therefore, it is not surprising that postmortem gene expression in this tissue initially is characterized by increased expression of anti-apoptotic genes, although eventually pro-apoptotic activity dominates [4]. Anti-apoptotic genes that demonstrate upregulated expression during certain time periods postmortem in the prostate, potentially mediating to some degree this gland’s relative resistance to decay, include BCL2, BIRC2, TNFSF5, and XIAP. Despite the observed gene expression differences between liver and prostate, the overall long-term trend for both organs is eventual dominance of pro-apoptotic pathways and outcomes, which distinguishes postmortem tissue from cancer cells exhibiting resistance to apoptosis.

Hypothesis

Cancer can be considered a form of “un-death,” a condition of excessively proliferating cells that are unusually resistant to apoptosis (programmed cell death) and subject to strong selective pressure for

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“uncontrolled life.” Our hypothesis is that if expansion of neoplastic cells is a key selective pressure driving cancer progression, then tissue-specific patterns of postmortem gene expression (i.e., the expression associated with organismal death) are incompatible with the ability of cancer for uncontrolled proliferation (“excessive life”). Thus, our hypothesis has three key components: (a) there will be distinct and opposing patterns of gene expression between the postmortem state and cancer with respect to key physiological outputs such as growth, apoptosis, invasion, and relative cancer prognosis; (b) cancer cells considered more aggressive (e.g., derived from a metastasis and/or resistant to agents that suppress growth or induce apoptosis) will exhibit expression of relevant genes more unlike that of the postmortem condition while less aggressive neoplastic cells will exhibit gene expression more similar to the postmortem condition; and (c) targeting gene expression in cancer to produce a more postmortem-like pattern will promote less tumorigenic and less aggressive cell phenotypes.

Evaluating the hypothesis

Initial evaluation of the hypothesis part I: using previously published gene expression data, postmortem mice vs. human neoplastic colonic cells

As cancer researchers with an interest in gene expression in colorectal cancer (CRC), we first determined correlations between postmortem expression in the mouse, an experimental model frequently utilized in CRC research, and our published microarray data from colonic neoplastic cell lines [6-9], including colon microadenoma, primary carcinoma, metastatic carcinoma, and butyrate-resistant derivatives of primary carcinoma. We subsequently compared this microarray data to findings from studies of postmortem gene expression in human cadaver tissue.

Neoplastic colonic cell lines from which gene expression data were generated for the aforementioned published studies included metastatic SW620 CRC cells, which were compared to LT97 colon microadenoma (very early stage neoplasia) cells; and primary CRC HCT-116 cells, which were compared to the p300 knockout (KO) HCT-116 cell lines (D10 and F5) that exhibit some degree of butyrate resistance [9]; and HCT-R cells that are resistant to butyrate and other histone deacetylase inhibitors (HDACis) with respect to effects on Wnt signaling [10-12] modulation, apoptosis, and suppressed growth. We note that physiological concentrations of butyrate, a fermentation product of dietary fiber in the colon, affects Wnt signaling, represses growth, and induces apoptosis of CRC cells [10-12]. Resistance to butyrate may be associated with greater tumorigenicity (i.e., aggressiveness) and possibly contributes to the development of human cancer [7-14].

Several genes with upregulated expression after death in mice also exhibit altered expression in neoplastic colonic cells. BCL6 and KISS1 that are upregulated postmortem in mice exhibit decreased expression in metastatic carcinoma SW620 cells compared to LT97 microadenoma cells [7]. In addition, BCL6 and KISS1 are downregulated in butyrate-resistant HCT-R cells compared to the butyrate-sensitive HCT-116 parental line [8]. Therefore, two genes with upregulated expression in postmortem murine tissue exhibit downregulated expression in the more aggressive metastatic (SW620) and drug-resistant (HCT-R) CRC cells, generally consistent with our hypothesis that patterns of postmortem gene expression are incompatible with carcinogenesis.

However, BCL6 more typically tends to be upregulated during colonic neoplastic progression [15], which differs from what is observed in the cell lines we analyzed. It is possible that the Wnt signaling status of the CRCs in question may influence BCL6 expression, as we have previously identified BCL6 as a gene upregulated by butyrate in a Wnt-dependent manner [8]. KISS1, whose product is associated with cancer invasion, migration, metastasis and angiogenesis [16] is more highly expressed in normal than in neoplastic tissue, although with respect to CRCs, it is expressed to a greater extent in larger tumors. Thus, while the trend of BCL6 and KISS1 expression in the examined cell lines supports our hypothesis, the overall expression data in CRC progression is more equivocal.

Despite being typically observed as a gene whose expression is downregulated in cancer [17], the pro-apoptotic gene BCL2L11 (BIM) is upregulated in SW620 as compared to LT97 cells, and in HCT-R as compared to HCT-116 cells (in the absence of butyrate) [7,8]. Unlike the observations for BCL6, BCL2L11 (BIM) is upregulated in postmortem mice and in the more aggressive neoplastic cells; however, the cancer expression data supporting the downregulation of BCL2L11 expression [17] are consistent with our hypothesis. BCL2L11 (BIM) has been shown to be a tumor suppressor in mouse models of leukemia [18], consistent with its pro-apoptotic function.

Other genes overexpressed in postmortem mouse tissues also have interesting correlations to expression in neoplastic colonic cells. LMO4, a gene whose expression can be upregulated in breast cancer and contributes to disease progression [19], is upregulated in SW620 cells compared to LT97 microadenoma cells. KAT7 and GRK4 are downregulated in HCT-R cells compared to HCT-116 cells; KAT7 gene expression pattern was observed in the absence of HDACi (butyrate) treatment, and GRK4 gene pattern was examined after butyrate treatment [8]. BCL6, BCL2L11 (BIM), and GRK4 were identified as genes whose expression was upregulated by butyrate treatment in HCT-116 in a Wnt activity-dependent manner [8]. KAT7 has been found to be mutated in various cancers, including colorectal cancer, potentially affecting histone acetylation and, hence, gene expression [20]. GRK4 expression has previously been associated with breast tumorigenesis [21].

Based upon the correlations between postmortem gene expression in mice and those observed in a variety of human neoplastic colonic cells, our hypothesis is only partially supported. However, these are cross-species comparisons, mouse to human, and this species difference may affect the correlations. Therefore, we proceeded to compare gene expression data of human colonic neoplastic cells to that obtained from postmortem human tissues.

Initial evaluation of the hypothesis part II: human postmortem data vs. human neoplastic colonic cells

First, we considered those genes upregulated postmortem in the myocardium, pericardial fluid, and blood of human cadavers [2]. Two of these genes, VEGFA and MMP9, exhibit differential expression in our data analyses of neoplastic cells [6-9]. VEGFA encodes an angiogenic factor with a role in bone and sperm development [22,23], and is linked to cancer metastasis [24]. VEGFA expression is upregulated by Wnt activity in breast cancer cells [25] and is downregulated in butyrate-treated SW620 cells compared to butyrate-treated LT97 microadenoma cells. It is similarly downregulated in HCT-R cells compared to HCT-116 cells in the absence of butyrate. VEGFA expression is upregulated in F5 p300 KO HCT-116 cells compared to wild-type HCT-116; however, it is downregulated in D10 HCT-116 p300 KO line. MMP9 is a Wnt target gene [26] involved in wound healing [27], whose expression is correlated to worse outcomes in CRC [28]. The expression of MMP9 is downregulated in butyrate-treated HCT-R cells compared to similarly treated HCT-116 cells and is also downregulated in both the F5 and D10 p300 KO lines.

Thus, in support of our hypothesis, expression of VEGA and MMP9 is upregulated in postmortem human samples, but downregulated in the more aggressive metastatic SW620 CRC cells compared to early stage LT97 microadenoma cells [7], and in butyrate-resistant HCT-R vs. parental butyrate-sensitive HCT-116 cells [8]. MMP9 is also downregulated in both p300 KO cell lines, while VEGFA is downregulated in D10 p300 KO but not F5 p300 KO cells [9]. Since the KO lines are partially butyrate-resistant and can be considered more aggressive forms of the HCT-116 line, these latter findings partially support our hypothesis as well. In general, the data support an inverse relationship between postmortem gene expression and gene expression observed in...
cells representative of greater tumorigenic potential (e.g., metastatic SW620 cells and butyrate-resistant HCT-R cells).

Our data reveal that the more aggressive cell lines (e.g., metastatic SW620 and butyrate resistant HCT-R) exhibit patterns of gene expression different than that observed in postmortem liver tissue. Thus, SW620 cells upregulate BIRC5 compared to LT97 microadenoma cells [7]; whereas, HCT-R cells upregulate BIRC5 and BCL2 compared to parental HDACi-sensitive HCT-116 [6]. The p300 KO lines upregulate BIRC5 compared to HCT-116 in the presence of butyrate [9]. Therefore, there is, ultimately, increased expression of pro-apoptotic genes post-mortem that contrasts with the enhanced expression of anti-apoptotic genes in CRC cells that exhibit greater aggressiveness, drug-resistance and/or potential for tumorigenicity.

**Further considerations of the comparative gene expression data for our hypothesis**

Postmortem gene expression likely represents a tightly controlled, tissue-specific process, rather than a sum of stochastic gene expression changes that occur after death. There is evidence of continuing regulation of gene transcription after organismal death, as well as some altered patterns of mRNA splicing [5], and it is also likely that post-mortem gene expression is affected by patterns of tissue-specific and gene-specific mRNA degradation that takes place after death [29].

Due to the tissue-specific nature of human postmortem gene expression there is a relatively low degree of overlap in the patterns of altered gene expression between different tissues [5]. Therefore, one possibility is that differences in gene expression patterns between more tumorigenic CRC cells and non-colonic postmortem tissues may in part be due to tissue-specific variations.

However, if observed differences in gene expression comparing postmortem non-colonic tissue and neoplastic colonic cells was truly only due to tissue-specific variation, then we would not expect to observe any consistent (negative) association between postmortem gene expression and colonic tumorigenicity. In that case, changes in gene expression observed postmortem would just as likely be to that observed with increased tumorigenicity as to be similar to that observed with lesser tumorigenicity.

For example, if postmortem tissue/neoplastic colonic cell differences in gene expression were only tissue-specific, then, e.g., postmortem liver tissue would be just as likely to be exhibit gene expression more similar to SW620 and HCT-R cells compared to LT97 and HCT-116 cells than vice versa. Although we currently have only limited data (see above), the data that do exist suggest the possibility that expression patterns in non-colonic postmortem tissue is regulated opposite to that of increased tumorigenicity (e.g. SW620 and HCT-R cells). If these preliminary findings are subsequently confirmed with more extensive and targeted analyses, then the differences in gene expression between postmortem tissue and CRC would be at least in part explained by differences between postmortem and neoplastic tissue.

In summary, the comparative analyses between microarray data of neoplastic colonic cells and human postmortem gene expression are generally and tentatively supportive of our hypothesis; however, these analyses do not provide definitive proof of the hypothesis. More data are required. An important first step would be to obtain detailed gene expression profiles from human postmortem colonic tissue.

**Future further testing of the hypothesis**

We hypothesize that the patterns of postmortem gene expression [1–5,29], associated with organismal death would be incompatible with the ability for uncontrolled proliferation (“excessive life”) of cancer. This is underscored by the relative enrichment in pro-apoptotic gene expression observed in human postmortem tissue [3,4]. We propose that postmortem patterns of gene expression can be informative in enhancing our understanding of cancer and for devising novel anti-cancer therapeutics. One approach for evaluating our hypothesis is to engineer postmortem patterns of gene expression in cancer cell lines and in transgenic mice. If possible, this will be accomplished in a tissue-specific manner [5], and engineering of the colonic cell lines will utilize information obtained from gene expression analyses of postmortem colonic tissue. Therefore, a necessary and important first step would be a comprehensive analysis of the postmortem gene expression patterns of the human colon and rectum, compared with that of both normal and neoplastic colorectal tissues, with a particular emphasis on those genes involved in key hallmarks of the colonic neoplastic phenotype.

Subsequently, the approach will entail upregulating the expression of a fraction of genes that exhibit increased expression in postmortem mice and human cadavers. Currently, it is not technically possible to create mice that recapitulate the entirety of postmortem gene expression. Cell culture experiments would be performed first and the findings thus generated would allow for a more targeted in vivo manipulation of postmortem gene expression in mouse models. The engineering of cell lines that encompass a significant portion of altered, postmortem-like gene expression would be a progressive, iterative process (see below).

In a similar fashion, genes with downregulated postmortem expression will be targeted in the engineered cell lines (e.g., with the CRISPR methodology).

The strategy (Fig. 1) will be to choose combinations of genes considered of greatest relevance, based, e.g., on known functions and crosstalk, correlations between expression patterns postmortem vs. these of living organisms, and on in vitro experiments determining functional consequences of altering the expression of a small subset of gene combinations in cell lines.

For instance, one can choose several genes in the categories of cancer, development, and apoptosis that are upregulated postmortem, and ascertain the functional consequences of concomitantly upregulating these in human cell lines under different experimental conditions. Promising leads will be validated in in vivo studies. In addition, findings from the in vitro experiments can be used as the basis for progressively engineering of human cell lines with an increasing number of sets of upregulated genes within the postmortem gene expression pattern. Engineering cells that mimic aspects of a death-specific gene expression pattern may lead to alterations of mRNA stability patterns observed postmortem [5], which would in turn identify other promising targets for genetic manipulation.

With respect to cancer, the engineered cell lines and mice will be
evaluated for their propensity to, or resistance to, tumorigenesis induced by pre-existing or engineered mutation, carcinogenic exposure, etc. The postmortem gene expression specifically associated with signaling disorders, and with the loss of proper control of integration with homeostasis, may enhance tumorigenesis; whereas, gene expression patterns associated with organismal death may induce resistance to tumorigenesis. The key selective pressure driving cancer progression is the genetic continuity and expansion of neoplastic cells, which likely leads to adaptive patterns of gene expression that are opposite to those seen with organismal death.

Conclusion

Comparative analyses of gene expression patterns in neoplastic cells with those of postmortem tissue suggest several fundamental similarities and differences. A propensity of cancer cells and postmortem tissue toward aberrant gene expression compared to normal cells in the living organism is a fundamental similarity; however, cancer is a form of “excessive life” (e.g., uncontrolled proliferation, resistance to apoptosis) which contrasts to the shutting down of organismal life functions at the cellular level postmortem. Therefore, it is expected that gene expression patterns in cancer and the postmortem state would reflect these underlying similarities and differences, and a survey of previously published data generally supports the hypothesis that greater tumorigenicity is associated with patterns of gene expression increasingly unlike that found in postmortem tissue, with a particular emphasis on the expression of genes involved in key characteristics of the neoplastic phenotype (e.g., apoptosis, invasion, etc.).

It is also anticipated that increased selection for a carcinogenesis-suppressive postmortem gene expression pattern of a particular tissue [x] would result in the engineering of cell lines and mice that are more cancer-resistant. Mechanisms of resistance could then be identified and evaluated. These mechanisms may become targets for novel preventive and therapeutic approaches against cancer. Based on currently available data [4, 5, 29], one would expect these therapeutic approaches to converge onto signaling pathways and patterns of gene expression that stimulate apoptosis. In addition, other, non-apoptotic, targets are expected to emerge from these analyses.

We note that whereas our data from studies with neoplastic colonic cells are not entirely representative of in vivo CRC cancers, these cell lines represent a practical model to evaluate the differences between neoplastic cell death program and postmortem gene expression. An important first step in correlating postmortem gene expression to CRC would be to obtain detailed gene expression data from human postmortem colonic tissue, to compare to that of normal and tumor tissue from living patients. Methodological analysis [30] has shown that postmortem human colon tissue can yield RNA of acceptable quality, in a range between that of liver and prostate, two tissues already utilized for human postmortem gene expression studies [3, 4].

Understanding the fundamental associations between postmortem and cancer gene expression can potentially lead to novel therapies aimed at inducing disorganization and death to cancer cells, while sparing normal cells. In addition, investigating postmortem gene expression can assist in the development of therapies for biological problems other than cancer, including the renewal of brain function at significant time intervals postmortem [31].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References