EXPLORATORY RESEARCH AND HYPOTHESIS IN MEDICINE

CONTENTS

Editorials

Cancer Dormancy: A New Hope
Surajit Sinha .............................................................. 1

Guillain-Barré Syndrome Following Viral Infections: Considerations for Future Treatment and Research
Lili Wang ................................................................. 4

Original Articles

Asthma Treatment: Role of Metabolism from a Double-blind Randomized Control Trial
Tarun Saxena, Sanjay Patidar, Manjari Saxena, Azeema Bhabrawala .................... 6

Differential Expression of Tight Junctions and Cell Polarity Genes in Human Colon Cancer
Themistoklis Kourkoumpetis, Kathryn E. Royse, Liang Chen, Milan Ravishankar, Michael Ittmann, Hashem B. El-Serag, Li Jiao .................. 14
Metastasis is the major cause of cancer-related deaths. Many patients develop metastatic relapse in secondary organs years after resection of the primary tumor, and usually succumb to the disease. The most likely explanation for this clinical outcome is that the early dissemination of tumor cells in the systemic circulation and seeding of secondary organs had occurred prior to the primary tumor resection and that disseminated tumor cells (DTCs) remained growth arrested in target organs for extended periods before the relapse. This asymptomatic phase of growth arrest before reactivation and manifestation of metastatic disease is called ‘tumor dormancy’.

Although most driver genetic alterations, like the RAS mutation (in pancreatic cancer), ERBB2 amplification (in breast cancer), androgen receptor amplification (in prostate cancer), can initiate primary tumor growth and dissemination, they are insufficient to drive metastatic outgrowth by themselves. Thus, during the dormancy phase, DTCs evolve, undergo additional molecular changes at both the genetic and epigenetic levels, engage or hijack differentiation and manifestation of metastatic disease. Since metastatic tumors are highly recalcitrant to chemotherapeutic drugs and are usually untreatable (eventually compromising the function of the organ), adjuvant treatments targeting DTCs or MICs during the dormancy phase can provide better therapeutic response and patient survival.

The DTC- or MIC-targeted approach provides the best window of opportunity for elimination of residual disease. However, understanding the biology of residual disease has been a challenge, mainly because of the lack of mouse models that can recapitulate the complex metastatic cascade of the human disease process. Previous studies have shown that targeted therapies or adjuvant treatments are largely ineffective in eliminating residual DTCs from the system because the biology and molecular signatures of disseminated disease is different from that of primary tumor, hinting at an indispensable need for development of mouse models of dormancy and metastasis.

A recent flurry of investigations using genetic screening in mice and dormant cell lines isolated from patients have provided some insights into the signaling mechanisms that govern metastatic dormancy and reactivation. This has raised and fostered hope that dissecting the biology and mechanisms that regulate dormancy and reactivation of tumor cells will lead to development of biomarkers that can aid in determining the extent of metastatic spread and in rationalizing patient treatment; in addition, it may also ultimately lead to improvement of existing treatments and to design of novel therapeutic modalities for eradication and complete remission of the cancer.

MICs arise from DTCs that detach from the primary tumor and enter the systemic circulation. Upon extravasation into a target organ, such as liver or lung, the majority of DTCs undergo rapid apoptosis and clearance because of mechanical/metabolic stresses and immune surveillance by the host. Few DTCs survive this attrition process and undergo extended growth arrest, and even fewer cells survive, gain competence and initiate metastatic outgrowth. As such, the entire metastatic cascade is a Darwinian selection process, wherein the majority of DTCs in the circulation and during extravasation are eliminated as they are not deemed fit. Only a very few survive the entire attrition process to develop into life-threatening lesions.

Emerging studies show that these few life-threatening lesions develop from cancer cells that have undergone reprogramming to become stem-like and exhibit high self-renewal properties that are just like normal somatic stem cells. Why DTCs become dormant and how they gain metastatic competence to re-initiate outgrowth at a distant organ is not known. The question arises then, can effective therapies be designed to eliminate disseminated residual cells? Such questions are only just beginning to be understood at the molecular level.

One hypothesis is that the absence of or reduced mitogenic signaling in the secondary organ can trigger tumor cells to undergo a G0/G1 arrest. In such scenarios, tumor cells are unable to engage growth factor signaling through integrin receptors and enter the state of dormancy or quiescence. Most organs in the human body have a growth-restrictive or rather dormancy-permissive microenvironment to any foreign tumor cell that has infiltrated the tissue. For example, in the bone marrow, growth arrest-specific protein 6 (GAS6) and specific bone morphogenetic protein (BMP7) can growth-arrest tumor cells and induce dormancy in leukemia and prostate cancers.

In the lungs, the abundance of BMP4, and similarly the anti-proliferative effect of transforming growth factor-beta 2 (TGF-β2), in the bone marrow can restrict the growth of DTCs and induce quiescence.

The second hypothesis involves the inability of tumor cells to mount an angiogenic switch. Without a constant supply of nutrients and in the absence of newly formed blood vessels, a balance is maintained between proliferation and apoptosis, leading to a state of dormancy. It has recently been theorized that dormancy is an active molecular program adopted by DTCs, just like quiescence of normal stem cells. This theory was supported by a study that demonstrated a significant overlap in the molecular programs that induce quiescence in hair and muscle stem cells with those of dormant head and neck carcinoma cells.

Recent investigations into the mechanisms of dormancy and
metastatic reactivation have demonstrated that dormant tumor cells activate intrinsic molecular programs that induce stem cell traits or self-renewal capacity to colonize a target organ. Moreover, genetic screening in mouse models and mechanistic work have identified tumor intrinsic factors and receptors that can cross-talk with the tumor microenvironment to initiate metastatic outgrowth.\textsuperscript{4,17,18}

A main question that persists is how understanding dormancy will improve patient treatment. First, the dissection of molecular mechanisms that dictate dormancy will greatly accelerate development of novel biomarkers that can distinguish early and late DTCs and generate dormancy gene signatures that can predict disease recurrence. Such markers can be used for monitoring metastatic progression. Adjuvant treatment of patients can then be stratified based on the risk of disease recurrence. Second, most conventional chemotherapeutic drugs are administered to induce cancer cell death but they also generate drug-resistant cells, which become untreated. In contrast, however, adjuvant treatments of patients with a cocktail of mitogenic signaling inhibitors can reprogram a metastatic initiating cell to enter perpetual dormancy. Third, dormant DTCs, which are supposedly cancer stem cells, although growth arrested in nature, have functional metabolically active survival pathways, such as autophagy, which can be targeted.

Understanding the metabolic and survival pathways at single-cell level will allow rational intervention to induce metastatic dormancy. It is, therefore, imperative to establish the genomic and proteomic profiles of dormant versus MICs to strategically intervene these processes. Thus, characterization of dormant and DTCs may not only be the best and quickest method of determining the timing of disease recurrence or extent of dormancy but may also help in rationalizing adjuvant treatment to delay the onset of recurrence or to drive DTCs into perpetual dormancy (Fig. 1).

Our knowledge on cancer dormancy is expanding fast, and application of this knowledge in the clinic is slowly budding. RNA sequencing technology at single-cell level has given a boom to this field and has helped in characterization of DTCs from bone marrow and circulating tumor cells from liquid biopsies. As most solid cancers are organotropic, it is the task of future scientists to develop organ-specific biomarkers for DTCs. It is also imperative to develop proteomic profiles of DTCs as proteins are the drivers of phenotype. Together, this knowledge will help in proper staging and stratification of cancer patients for adjuvant therapy. Although the field of cancer dormancy is currently in its infancy and many technological challenges remain, it holds great potential and promise for future cancer treatments and patient management, and if successful can establish a paradigm for next-generation treatment modalities.

References


Guillain-Barré Syndrome Following Viral Infections: Considerations for Future Treatment and Research

Lili Wang*

Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, NY, USA

As the world’s population grows in an explosive way, the circumstances we live in become more crowded than ever in human history. Although medicine and biotechnology have developed tremendously in the new century, the growing density of population has brought us unexpected challenges in public health. This not only causes the emergence of new viruses but also makes coinfection and super-infection more common than previously (Table 1). However, the symptoms of these infections, although severe and life threatening in extreme cases, share some common characteristics in outcomes. Many of them cause Guillain-Barré syndrome (GBS) and respiratory emergency. The case reported by Hariharan et al. has provided a good reference for the treatment outcomes of such conditions.

GBS is an autoimmune disorder, in which the immune system attacks the peripheral nervous system. It causes muscle weakness, due to damage to nerve cells and their supporting structures. Different types of GBS feature different types of immune attack. Although it is a relatively rare event, GBS could be life threatening, with bulbar and respiratory involvement. It is reported that in two-thirds of patients, neuropathic GBS occurs after an infection. GBS is a known sequelae of Dengue infection, and has been reported in influenza virus, herpesvirus and hepatitis virus infections, etc. Literally, any virus infection has the potential to cause GBS. Due to the rising pandemics of Zika virus in recent years, GBS has become a focus of discussion. In 2013–2014, GBS was reported from the areas of Zika virus outbreaks, and in 2015, reported again in Oceania and the Americas. Statistically, during Zika pandemics in French Polynesia, among 28,000 persons under medical care, 38 (0.14%) patients developed GBS, compatible with the acute motor axonal neuropathy subtype of the disease.

In recent years, virus-caused pandemics seem to have a trend of taking off, and the syndromes caused by these diseases have also showed some new and more complicating characteristics. One typical example is the reoccurrence of Zika virus. On its initial appearance in the 1950s, it seemed to be mild. As such, Zika virus infection was considered as benign throughout the 20th century. But, in the recent 2015 outbreak in Brazil, it was reported to be associated with GBS and microcephaly. Considering Dengue and Zika viruses are from the same Flavivirus family, it is not surprising that the symptoms of Zika infection are often confused with Dengue virus infection and Dengue-chikingunya super-infections when causing GBS.

In all the cases reported for the 2013–2015 Zika virus breakouts, the infected persons showed mild fevers, headaches and body pains. These symptoms are very similar to those of Dengue and chikingunya infections, two other viral diseases that are often endemic in the same areas and are also transmitted by Aedes mosquitoes. Indeed, this may have caused many Zika infections to be mistaken as Dengue or chikingunya. Considering the possibilities of super-infections of multiple viruses, it is very difficult to confirm that the associated GBS is due to which viral infection(s). Even though the example provided by this case report has significant clinical magnumence (without associated thrombocytopenia), the complication is very similar to concurrent Zika virus infection. Under specific conditions that cause respiratory emergency, the treatment used in this case report can be applied to GBS induced by Zika virus infection.

Although GBS is a rare event in virus infection, the lethal consequence needs proper treatment and that’s where we should provide corresponding care to the patients. In Table 1, the main viruses causing pandemics in recent years are listed. One thing worth noticing is that most of these viruses can cause GBS and/or respiratory emergency. As the emerging viruses like Zika virus have no proper vaccines to prevent infection, under a condition when there is a new virus pandemic without proper vaccines, the treatment of a severe lethal syndrome becomes the focus of emergency. This paper may provide some valuable references when coping with emerging viruses that can cause GBS and/or respiratory emergency.

Traditionally, the method to treat respiratory emergency usually involves surgical tracheostomy. In this case, percutaneous dilatational tracheostomy (PCT) was performed instead of classical tracheostomy. Compared to the old method, PCT has a smaller size of incision and quicker recovery. Also, PCT is supposed to be a bedside procedure that can be performed by every physician, with less assistance and material. These traits fit the requirements when there is a breakout of viruses and large numbers of patients need to be taken care of. PCT could be a potential lifesaving method when facing certain future emerging virus attacks that cause respiratory emergency. Another critical method used in this case report is IV-Ig immune therapy. Based on the response of the patients while the treatments were going on, it is clear that IV-Ig here plays a key role in the patient’s recovery. However, this is not surprising, for IV-Ig immune therapy is already a routine method to treat GBS clinically.

In summary, although this case report provided a specific condition of combined chikingunya and Dengue infection, it could become very typical in future virus pandemics. The successful treatment of this condition provided valuable reference for emerging virus pandemics that may require a similar treatment for GBS and life-threatening breathing problems.
Table 1. Recent virus pandemics and their relationship to Guillain-Barré syndrome and respiratory distress syndrome

<table>
<thead>
<tr>
<th>Outbreaks</th>
<th>Year</th>
<th>May cause Guillain-Barré syndrome</th>
<th>May cause respiratory distress syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe acute respiratory syndrome</td>
<td>2002–2003</td>
<td>Unknown</td>
<td>Yes1</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>2006</td>
<td>Yes2</td>
<td>Yes3</td>
</tr>
<tr>
<td>Zika virus in Yap Island, Federated States of Micronesia</td>
<td>2007</td>
<td>Yes4</td>
<td>Unknown</td>
</tr>
<tr>
<td>H1N1 influenza</td>
<td>2009–2010</td>
<td>Yes5</td>
<td>Yes6</td>
</tr>
<tr>
<td>Measles in Congo</td>
<td>2010–now</td>
<td>Unknown</td>
<td>Yes7</td>
</tr>
<tr>
<td>Middle East respiratory syndrome</td>
<td>2012–now</td>
<td>Unknown</td>
<td>Yes8</td>
</tr>
<tr>
<td>Zika virus in French Polynesia</td>
<td>2013–2014</td>
<td>Yes9</td>
<td>Yes10</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>2013–now</td>
<td>Yes2</td>
<td>Yes3</td>
</tr>
<tr>
<td>Ebola in West Africa</td>
<td>2013–now</td>
<td>Unknown</td>
<td>Yes11</td>
</tr>
<tr>
<td>Zika virus in Brazil and Colombia</td>
<td>2015–now</td>
<td>Yes12</td>
<td>Yes10</td>
</tr>
<tr>
<td>Dengue fever in Hawaii and tropical Asian islands</td>
<td>2015, 2017–now</td>
<td>Yes13</td>
<td>Yes14</td>
</tr>
</tbody>
</table>

References


DOI: 10.14218/ERHM.2017.00035 | Volume 3 Issue 1, March 2018
Asthma Treatment: Role of Metabolism from a Double-blind Randomized Control Trial

Tarun Saxena¹*, Sanjay Patidar², Manjari Saxena¹ and Azeema Bhabrawala¹

¹Mittal Hospital and Research Center, Pushkar Road, Ajmer, India; ²Ministry of Health and Family Welfare, Government of India

Abstract

Background and objectives: The role of metabolism (basal metabolic rate (BMR)) in asthma remains dubious. Seasonal variations are reported in both BMR and asthma, with the former increasing in winter and decreasing in summer. Correlation between metabolism and asthma treatment is not obvious in the literature; therefore, it was planned to assess role of substances which alter metabolism in managing asthma.

Methods: This was a randomized double-blind active control trial, in which 400 confirmed asthma cases were divided into two groups (group 1 (control) and group 2 (study)), with group 2 further subdivided into two (subgroups 2A and 2B). The study period was from January to December 2016. During attack, group 2A was given kitchen spices (cloves, bay leaf, black pepper; substance A) and 2B was given cool substances (rose petal jam; substance B). The control group was given inhaled levosalbutamol. Symptoms and peak expiratory flow rate (PEFR) were recorded. Improvement was suggested by reduction in symptoms and improvement in PEFR. Review of symptoms was done at 24 and 72 h and of PEFR at 72 h after the therapy. Statistical significance of differences between group 1 and 2A and 2B was calculated by the chi-square test by using SPSS 20 software.

Results: Exacerbations were found in February (season: winter), March (spring), August (rainy) and October (autumn). In February and March, the response was significant with substance A ($p < 0.001$), but substance B had poor response. In August and October, the response was significant with substance B ($p < 0.001$), and substance A had insignificant response.

Conclusions: Substances which increase BMR are helpful in winters and high metabolic state of the body, and conversely. Change in metabolism possibly effects heat and water losing/preserving effect of the body, including related to the respiratory tract (thermoregulation), and lessens inflammation in the respiratory tract.

Introduction

Incidences of bronchial asthma are mounting. There are multiple risk factors that have been found to be associated with asthma,¹–⁵ and the role of metabolism in asthma is known to be complex. However, the correlation between metabolism and asthma treatment is less clearly described in the literature. A few studies have shown high basal metabolic rate (BMR), or metabolism, in asthmatic patients, while others have found that obesity and asthma are associated with each other.⁶,⁷ Seasonal variations in asthma and BMR have also been reported. Some studies have depicted a higher incidence of asthma during cold weather,⁸–¹¹ while others have suggested a higher incidence in summer and humid environment.¹²,¹³ According to Ayurveda, the BMR/metabolism of each cell/systemic changes across different seasons; during winter, the BMR increases and the body preserves heat,¹⁴–¹⁶ on the contrary, during summer, the BMR decreases and the body releases heat.¹⁴,¹⁷,¹⁸ Various kitchen spices, such as cloves,¹⁹,²⁰ have been associated with an increase in BMR, while substances like rose petal jam have been found to be associated with decrease in BMR.²¹,²² To assess whether seasonal changes in asthma and metabolism are interrelated in this study, various substances that affect metabolism were administered. Therefore, the study was planned to see the effect of kitchen spices/rose petal jam on bronchial asthma treatment.
Methods

Study design

This study was a randomized double-blind, interventional, parallel group, active control trial (CTRI/2016/12/007589), and was conducted in the Department of Internal Medicine and the Department of Yoga and Ayurveda. Patients came from the city and nearby villages encompassing an area of 20–25 kilometers and representing a population of 2,000,000.

Case selection, study period and study methods

Arbitrarily, a sample of 400 confirmed cases of bronchial asthma regularly attending to the hospital and presenting mild asthma symptoms during that prior 2 years were selected for the study. Informed written consent was obtained from each participant upon study enrollment and approval of the institutional ethical committee was awarded. Initial diagnosis was based on FEV1, forced expiratory volume in one second; of <80% and reversibility (increase in FEV1 >12% after 20 m of two levosalbutamol puffs).

Patients having a history of thyroid disorders, smoking, ischemic heart disease, hypertension, diabetes, requirement for oxygen therapy, moderate to severe symptoms of asthma, chronic respiratory infections, anemia and/or working in industries were excluded. The included cases were randomly divided into 2 groups, with group 1 (n = 200) representing the controls and group 2 representing the interventional group (n = 200). Group 2 was then subdivided randomly into group 2A (n = 100) and group 2B (n = 100).

The age range of the selected cases was 15–45 years-old, and the study population included both males and females. The patients were studied from January 2016 to December 2016. The symptoms and PEFR, Peak Expiratory Flow Rate) were recorded by a specific technician in the hospital and checked by a consultant on that same day. The following parameters were considered in each case (monthly): symptoms and PEFR; exposure history to any aeroallergen (pollens or flowers) or festival allergen (smoke, paint); occupational exposure; history or presence of viral respiratory infections; and, exposure to various environmental conditions.

A record of environmental factors (air temperature, humidity, air velocity, presence of any dust storm) throughout the 12-month period was obtained from the meteorological department. A record of viral and bacterial respiratory infections, and epidemics was obtained from the Chief Medical Health Officer’s office and the hospital record.

Symptom scoring

A normalized pattern was used. Four symptoms were included: cough; breathlessness; wheezing; and, chest heaviness. Each symptom was counted as one. For the attack, a symptom score of >2 with reduction in PEFR 15–20% was considered as an ‘attack’. The attack rate was calculated monthly by taking the number of attacks each month and dividing by the total number of cases.

Intervention

The intervention was given during the high attack rate period, when >50% of subjects had an attack. Group 2A was given substance A, which produces warmth inside the body (hot substances) and consisted of a mixed powder of kitchen spices, specifically two cloves (Syzygium aromaticum), 1 tejpatra leaf (Indian bay leaves) and 2 black pepper corns (Piper nigrum), given twice daily. Group 2B was given substance B, which produces coolness inside the body (cool substances) and consisted of 1 Tablespoon of gulkand (sweet preserve of rose petals, known as petal jam) and 2 teaspoons of syrup of Brahmi (Bacopa monnieri) in a glass of water mixed with 10 g of Mishri (crystallized sugar lump), given twice daily. Comparison was done between each subgroup and the control group, which was given inhaled levosalbutamol (50 mcg by two puffs thrice daily) at the time of attack. The process was repeated during each attack throughout the 12-month study period. The cases were strictly monitored over a course of 72 h to detect any worsening of symptoms.

Outcome measurement

Review of the symptoms of asthma cough, wheezing, chest heaviness and breathlessness at 24 and 72 h and of PEFR at 72 h after the therapy, and comparisons with the control group were done at the same time.

Statistical analysis

The collected data were entered in a Microsoft Excel sheet. Statistical analysis was carried out by using SPSS 20.0 statistical software. The appropriate test of significance was applied (chi-square test).

Results

The baseline features of group 1 and subgroups 2A and 2B were
Comparability (Table 1). There were various allergens during the different months (Table 2). The maximum asthma exacerbations occurred in February, March, August and October (Table 3). Irrespective of allergens during February and March, a significant response (both of symptoms and PEFR) to the intervention was found for subgroup 2A compared to group 1 ($p < 0.001$). During August and October, a significant response (both of symptoms and PEFR) to the intervention was found for subgroup 2B compared to group 1 ($p < 0.001$) (Tables 4 and 5; Fig. 1).

**Discussion**

Worldwide, incidence of asthma is on the rise. The role of metabolism in asthma remains unclear. A few studies have shown high BMR (metabolism) in asthma patients. Seasonal variations in asthma and BMR are also reported. Still, a clear relationship between asthma and metabolism remains to be established. Therefore, this study was planned to observe seasonal variations of asthma and the effect of various substances affecting metabolism on the clinical profile of asthma.

As already detailed in the Methods section, the study was done in a medical institute, with 400 confirmed cases of asthma, and lasted from January 2016 to December 2016. The cases were randomly divided into two groups, including group 1 (control, $n = 200$) and group 2 (study). The group 2 was further subdivided into group 2A ($n = 100$) and group 2B ($n = 100$). Through the 12-month study period, the symptoms and PEFR were recorded. Allergen exposure history was also recorded. Attack/exacerbation was considered by an increase in the symptoms and a reduction in PEFR. The intervention was given during the high attack rate period, when >50% subjects had an attack, and consisted of giving substance A to group 2A (mixed powder of kitchen spices) and substance B to group 2B (combination of substances like gulkand or preserve of rose petals, known as petal jam). Substance A is a mixture which increases BMR (hot substances), while substance B is a combination of substances which reduce BMR (cool substances).

Results in subgroups 2A and 2B were compared at 24 and 72 h for symptoms and PEFR with group 1, which had been given inhaled levosalbutamol.

As already detailed in the Results section, in the present study, four periods of high attack rate were found, one in February (winter), the second in March (spring), the third in August (rainy season), and the fourth in October (onset of winter or autumn). During the rest of the year, there was not much increase in attack rate. The attack rate was insignificant during peak summer (May and June) and peak winter (December and January). During February and March, a significant response (both symptoms and PEFR) to the intervention was found for group 2A compared to group 1. In August and October, a significant response (both symptoms and PEFR) to the intervention was found for group 2B as compared to group 1.

Thus, during winter and spring months, the response was better with substances that increase metabolic rate and produce warmth inside the body (such as substance A), similar to findings in other studies, and during summer and autumn, the response was better with substances that reduce metabolic rate and give a cooling effect to the body (such as substance B).

On the contrary, during the winter months and spring, the response to substance B was poor; however, this was found to be effective during the rainy season and autumn. Similarly, substance A was ineffective during the rainy season and autumn, but was found to be effective during winter and spring. Environmental temperature is low during the winter and spring and high during the rainy season and autumn. The metabolic rate is high in low environmental temperature and low in high environmental temperature (Fig. 2). This suggests that response to a particular substance (A or B) is season- or body metabolism-dependent and not a direct effect nor immunity-dependent. It, therefore, indicates a possible role of metabolism in management of asthma. It also suggests that metabolism in spring continues to be that of winter metabolism, and in autumn continues to follow summer metabolism.

BMR is the minimum cellular activity required for survival in a particular season. For an individual, the BMR primarily depends on height, weight, age and sex, but it changes with season. BMR increases in cold temperature and decreases in summer (i.e. basal activity of each cell necessary for its survival changes according to season). This is also depicted in Ayurveda. As shown in Figure 2, the hypothalamus senses environmental temperature and sets BMR/metabolism accordingly. Metabolism of the body sets a particular temperature/season after exposure over a period of time, and not instantly.

The high attack rate in our study was attributed to different allergens in different months. Besides other factors in February (66% viral infections) and August (74% viral infections), the maximum contribution was accounted for by viral infections; in March and October, despite exposure to coloring agents, smoke and crackers (30–35% contribution), the maximum effect was of change in tem-
perature, with almost 100% of cases being exposed to the external environmental temperature. In this part of the country there is a change of season in March (spring), that starts off as an increase in the environmental temperature (19–27 °C maximum temperature from February to March), but in September and October (autumn) it starts off as a decline in the environmental temperature (30–22 °C minimum temperature from September to October). This increase and decrease in environmental temperature opposes the ex-

**Table 3. Symptoms and PEFR before intervention, for January–December 2016**

<table>
<thead>
<tr>
<th>Month</th>
<th>Group</th>
<th>Cough</th>
<th>Breathlessness</th>
<th>Wheezing</th>
<th>Chest heaviness</th>
<th>PEFR reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>1</td>
<td>16%</td>
<td>22%</td>
<td>23%</td>
<td>12%</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>18%</td>
<td>22%</td>
<td>21%</td>
<td>13%</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>17%</td>
<td>22%</td>
<td>21%</td>
<td>11%</td>
<td>12%</td>
</tr>
<tr>
<td>February</td>
<td>1</td>
<td>93%</td>
<td>95%</td>
<td>92%</td>
<td>75%</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>93%</td>
<td>96%</td>
<td>94%</td>
<td>71%</td>
<td>92%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>95%</td>
<td>91%</td>
<td>91%</td>
<td>73%</td>
<td>91%</td>
</tr>
<tr>
<td>March</td>
<td>1</td>
<td>94%</td>
<td>93%</td>
<td>94%</td>
<td>72%</td>
<td>93%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>94%</td>
<td>92%</td>
<td>94%</td>
<td>73%</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>94%</td>
<td>93%</td>
<td>94%</td>
<td>71%</td>
<td>95%</td>
</tr>
<tr>
<td>April</td>
<td>1</td>
<td>24%</td>
<td>21%</td>
<td>17%</td>
<td>18%</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>23%</td>
<td>20%</td>
<td>16%</td>
<td>18%</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>24%</td>
<td>21%</td>
<td>16%</td>
<td>18%</td>
<td>16%</td>
</tr>
<tr>
<td>May</td>
<td>1</td>
<td>12%</td>
<td>11%</td>
<td>8%</td>
<td>9%</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>12%</td>
<td>10%</td>
<td>12%</td>
<td>11%</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>12%</td>
<td>10%</td>
<td>9%</td>
<td>9%</td>
<td>10%</td>
</tr>
<tr>
<td>June</td>
<td>1</td>
<td>14%</td>
<td>10%</td>
<td>8%</td>
<td>8%</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>16%</td>
<td>11%</td>
<td>9%</td>
<td>8%</td>
<td>11%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>15%</td>
<td>11%</td>
<td>8%</td>
<td>8%</td>
<td>11%</td>
</tr>
<tr>
<td>July</td>
<td>1</td>
<td>42%</td>
<td>35%</td>
<td>28%</td>
<td>21%</td>
<td>32%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>40%</td>
<td>36%</td>
<td>28%</td>
<td>21%</td>
<td>32%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>40%</td>
<td>36%</td>
<td>28%</td>
<td>21%</td>
<td>32%</td>
</tr>
<tr>
<td>August</td>
<td>1</td>
<td>95%</td>
<td>94%</td>
<td>95%</td>
<td>83%</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>96%</td>
<td>95%</td>
<td>95%</td>
<td>77%</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>95%</td>
<td>96%</td>
<td>95%</td>
<td>77%</td>
<td>95%</td>
</tr>
<tr>
<td>September</td>
<td>1</td>
<td>13%</td>
<td>14%</td>
<td>17%</td>
<td>9%</td>
<td>13%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>15%</td>
<td>14%</td>
<td>15%</td>
<td>9%</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>13%</td>
<td>14%</td>
<td>16%</td>
<td>9%</td>
<td>12%</td>
</tr>
<tr>
<td>October</td>
<td>1</td>
<td>96%</td>
<td>96%</td>
<td>94%</td>
<td>70%</td>
<td>92%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>95%</td>
<td>95%</td>
<td>96%</td>
<td>72%</td>
<td>93%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>96%</td>
<td>95%</td>
<td>93%</td>
<td>70%</td>
<td>93%</td>
</tr>
<tr>
<td>November</td>
<td>1</td>
<td>41%</td>
<td>44%</td>
<td>38%</td>
<td>22%</td>
<td>37%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>43%</td>
<td>38%</td>
<td>39%</td>
<td>21%</td>
<td>38%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>45%</td>
<td>44%</td>
<td>39%</td>
<td>21%</td>
<td>33%</td>
</tr>
<tr>
<td>December</td>
<td>1</td>
<td>22%</td>
<td>18%</td>
<td>16%</td>
<td>12%</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>22%</td>
<td>18%</td>
<td>18%</td>
<td>11%</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>24%</td>
<td>19%</td>
<td>18%</td>
<td>12%</td>
<td>16%</td>
</tr>
</tbody>
</table>
existing metabolism of the body in March, in which a state of existent high metabolism is opposed by rising environmental temperature which tries to bring down the metabolism. During October, a state of existent low metabolism is opposed by declining environmental temperature which tries to raise the metabolism; therefore, the asthma attack rate is high during this period.

Table 4. Symptoms and PEFR after intervention, for January–December 2016

<table>
<thead>
<tr>
<th>Symptom and PEFR group</th>
<th>February After 24 h</th>
<th>March After 72 h</th>
<th>August After 24 h</th>
<th>October After 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improvement in &gt;2 symptoms</td>
<td>60%</td>
<td>84%</td>
<td>68%</td>
<td>82%</td>
</tr>
<tr>
<td>Improvement in PEFR</td>
<td>82%</td>
<td>80%</td>
<td>64%</td>
<td>64%</td>
</tr>
<tr>
<td>Symptoms and PEFR group 2A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement in &gt;2 symptoms</td>
<td>78%</td>
<td>96%</td>
<td>84%</td>
<td>97%</td>
</tr>
<tr>
<td>Improvement in PEFR</td>
<td>94%</td>
<td>96%</td>
<td>14%</td>
<td>17%</td>
</tr>
<tr>
<td>Symptoms and PEFR group 2B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement in &gt;2 symptoms</td>
<td>12%</td>
<td>16%</td>
<td>15%</td>
<td>16%</td>
</tr>
<tr>
<td>Improvement in PEFR</td>
<td>15%</td>
<td>14%</td>
<td>86%</td>
<td>94%</td>
</tr>
</tbody>
</table>

Abbreviation: PEFR, peak expiratory flow rate.

Table 5. Significance level

<table>
<thead>
<tr>
<th>Groups Symptoms</th>
<th>February 24 h/72 h</th>
<th>March 24 h/72 h</th>
<th>August 24 h/72 h</th>
<th>October 24 h/72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs 2A p-value</td>
<td>0.003/&lt;0.001</td>
<td>&lt;0.001/&lt;0.001</td>
<td>&lt;0.001/&lt;0.001</td>
<td>&lt;0.001/&lt;0.001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>0.219/0.191</td>
<td>0.141/0.098</td>
<td>9.913/11.61</td>
<td>22.382/26.21</td>
</tr>
<tr>
<td>1 vs 2B p-value</td>
<td>&lt;0.001/&lt;0.001</td>
<td>&lt;0.001/&lt;0.001</td>
<td>&lt;0.001/&lt;0.001</td>
<td>&lt;0.001/&lt;0.001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>27.56/32.94</td>
<td>23.91/26.46</td>
<td>0.085/0.082</td>
<td>0.162/0.150</td>
</tr>
<tr>
<td>2A vs 2B p-value</td>
<td>&lt;0.001/&lt;0.001</td>
<td>&lt;0.001/&lt;0.001</td>
<td>&lt;0.001/&lt;0.001</td>
<td>&lt;0.001/&lt;0.001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>126.0/137.2</td>
<td>169.7/174.3</td>
<td>0.009/0.008</td>
<td>0.007/0.007</td>
</tr>
<tr>
<td>1 vs 2 p-value</td>
<td>&lt;0.001/&lt;0.001</td>
<td>&lt;0.001/&lt;0.001</td>
<td>0.03/&lt;0.001</td>
<td>&lt;0.001/&lt;0.001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>4.12/4.16</td>
<td>3.50/3.67</td>
<td>1.56/1.62</td>
<td>3.80/3.78</td>
</tr>
<tr>
<td>PEFR 1 vs 2A p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>0.038</td>
<td>0.040</td>
<td>8.84</td>
<td>4.16</td>
</tr>
<tr>
<td>1 vs 2B p-value</td>
<td>0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>3.436</td>
<td>5.988</td>
<td>0.0993</td>
<td>0.449</td>
</tr>
<tr>
<td>2A vs 2B p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>88.71</td>
<td>147.42</td>
<td>0.011</td>
<td>0.011</td>
</tr>
<tr>
<td>1 vs 2 p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.053</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>3.8</td>
<td>3.27</td>
<td>1.484</td>
<td>3.57</td>
</tr>
</tbody>
</table>
Fig. 1. Response to asthma symptoms. A good response to cool substances during state of low metabolism, and conversely, occurs.

Winter/Spring
- High Metabolic State
- Heat Preservation in Body & Respiratory Tract
- Viral Exposure/Increase in Environmental Temperature
- Opposes/Alters Existing Metabolism
- Heat/Water Release in Body & Respiratory Tract
- Inflammation in Respiratory Tract (Asthma Symptoms)
- Hot Substance (clove)
- Increase in Metabolism (reverting back to previous metabolism)
- Heat Preservation in Body & Respiratory Tract
- Inflammation Subsides in Respiratory Tract
- Correction in Asthma Symptoms

Summer/Autumn
- Low Metabolic State
- Heat & Water Release in Body & Respiratory Tract
- Viral Exposure/Decrease in Environmental Temperature
- Opposes/Alters Existing Metabolism
- Heat/Water Preservation in Body & Respiratory Tract
- Inflammation in Respiratory Tract (Asthma Symptoms)
- Cool Substance (rose petal jam)
- Decrease in Metabolism (reverting back to previous metabolism)
- Heat/Water Release in Body & Respiratory Tract
- Inflammation Subsides in Respiratory Tract
- Correction in Asthma Symptoms

Fig. 2. State of metabolism during different months set by hypothalamus. The state of low metabolism in summer months and high metabolism in winter months.

Fig. 3. Flow chart of the asthma/season changes.
All allergens produce inflammation of respiratory mucosa, clinically presenting as cough wheezing, breathlessness and chest pain associated with reduction in PEFR.\textsuperscript{26,27} Correction in metabolism (i.e., metabolism suitable to particular season) possibly reduces inflammation in respiratory mucosa and, therefore, improves asthma symptoms. This is irrespective of allergen exposure, especially because attacks post-viral infection in February (66\%) and August (74\%) were quickly corrected by substance A and substance B respectively in environments suitable to the substance.

In summary, we found that metabolism of the body changes with environmental temperature and season. Specifically, in winter and spring, metabolism remains high and asthma symptoms respond well to substances which increase the metabolic rate, such as clove. In summer and autumn, metabolism remains low and asthma symptoms respond well to substances which reduce the metabolic rate, like rose petal jam.

The possible mechanism by which change in metabolism affects inflammation in the tracheobronchial tree seems to involve the respiratory tract, as one of the sites of heat and water release and thermoregulation in the body.\textsuperscript{28,29} and changes in heat release which affect inflammation in the respiratory tract.\textsuperscript{29,30} Changes in heat release or heat preservation are parallel to the heat-releasing or heat-saving mechanisms of the body. In low environmental temperature or the high metabolism state,\textsuperscript{14,15} heat preservation occurs throughout the body, including in the respiratory tract. This prevents inflammation in the respiratory tract. A sudden increase in external temperature (environmental or inhaled) gives impulses to the hypothalamus, opposite to the existing metabolism of heat preservation. There is a sudden slowdown of metabolism and release of heat throughout the body, including in the respiratory tract. This produces an inflammatory effect in the respiratory tract and nasal mucosa.\textsuperscript{31–33}

By administering clove-like substances, metabolism is increased, correction of metabolism occurs and inflammation in the respiratory tract subsides, and vice versa. This is similar to a person wearing a warm jacket in a cold environment. The person is comfortable; however, if s/he suddenly experiences heat due to sunlight s/he will face uneasiness and will perspire. A similar uneasiness is present in the respiratory tract (both skin and respiratory tract are heat-releasing sites) and are associated with inflammation and asthma symptoms. Once s/he removes the jacket and comes back to the cold environment, s/he becomes comfortable in a way that is suitable to body metabolism. Quick response, even at 24 h, almost excludes all other possibilities of improvement, including modulation of immunity. In viral infection, though portal of entry is the respiratory tract, the virus produces systemic symptoms like fever, myalgia, exanthem and bronchospasm, which affect metabolism and therefore can be helped by substances favorable to seasonal metabolism (Fig. 3).

Hypothesis and future research direction

It is hypothesized that management of asthma is metabolism-dependent. Hot substances like clove are helpful in regressing asthma symptoms in cold weather or high metabolic state, and cool substance like rose petal jam are helpful in mitigating asthma symptoms in warm weather or low metabolic state. Alteration in metabolism possibly affects heat/water losing/preserving effect of the body, including in the respiratory tract (thermoregulation) and reduces inflammation in the respiratory tract. Body and lungs function smoothly in a metabolic state which is most suitable to the environment. Further studies are required to elucidate the association of the BMR-increasing and -decreasing substances with seasonal changes in treatment of asthma.

Conclusions

Management of asthma is metabolism-dependent. It bears repeating, hot substances like clove are helpful in regressing asthma symptoms in cold weather or high metabolic state, and cool substance like rose petal jam are helpful in mitigating asthma symptoms in warm weather or low metabolic state. Alteration in metabolism possibly affects heat/water losing/preserving effect of the body, including in the respiratory tract (thermoregulation), and reduces inflammation in the respiratory tract. Body and lungs function smoothly in a metabolic state which is most suitable to the environment.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study design (TS, SP), manuscript writing (TS, MS); data collection (SP, MS), data analysis (SP), counseling the patients (MS, AB), collection and editing of references (AB).

References


DOI: 10.14218/ERHM.2017.00030 | Volume 3 Issue 1, March 2018 13
**Differential Expression of Tight Junctions and Cell Polarity Genes in Human Colon Cancer**

Themistoklis Kourkoumpetes1,2, Kathryn E. Royse3, Liang Chen1,2,3, Milan Ravishankar1,2, Michael Ittmann2,4, Hashem B. El-Serag1,2,3 and Li Jiao1,2,3*

1Section of Gastroenterology and Hepatology, Baylor College of Medicine, Houston, USA; 2Michael E. DeBakey VA Medical Center, Houston, Texas, USA; 3Section of Health Service Research, Department of Medicine, Baylor College of Medicine, Houston, USA; 4Department of Pathology and Immunology, Baylor College of Medicine, Houston, USA

Abstract

**Background and objectives:** Tight junction and cell polarity proteins are paramount to epithelial cell polarity and transport. *In vivo* studies have shown the differential expression of tight junctions in colorectal cancer, with little to no human data to corroborate those findings. We investigated whether tight junction genes were differentially expressed in human colon cancer versus normal controls.

**Methods:** Total RNA was extracted from fresh frozen tumor tissues and normal adjacent tissues of 6 patients who were diagnosed with primary colon cancer as well as from normal mucosa of 5 unrelated polyp-free individuals. We used the Qiagen RT2 Profiler PCR array to determine the expression of 84 genes in the tight junction pathway. Student’s t-test was used to compare gene expression levels between cancer tissues and normal mucosa using normalized gene expression data.

**Results:** Compared with normal mucosa, significant up-regulation of the claudin 1 (*CLDN1*) gene (fold-change = 16, *p* = 0.001) but down-regulation of the *AMOTL1, CLDN5, JAM2* and *TIAM1* genes (fold-change > 2, *p* < 0.05) were seen in colon cancer tissue.

**Conclusions:** We observed the differential expression of *CLDN, AMOTL1, JAM2* and *TIAM1* in colon cancer versus normal mucosa. Further larger studies are warranted to investigate the role of tight junction and cell polarity proteins in the progression of human colon tumors.

Introduction

The intestinal epithelium is a selective semipermeable barrier for luminal molecules and microbes. It maintains its complexity through a diverse family of molecules that promote cell-to-cell anchorage and appropriate individual cell polarity, mainly being represented by the adherens junctions and tight junctions. Tight junctions are a diverse family of molecules which form an essential barrier between the internal and external environment and maintain its complexity.

Cell-to-cell adhesion is paramount to the maintenance of normal epithelial tissue architecture, and disruption of this anchorage is believed to aid to tumor progression and subsequent invasion to deeper tissues as well as distant metastasis. Intestinal barrier dysfunction is also an essential contributor to intestinal inflammation. Structural disruption of colonic epithelial cells might impact early tumorigenesis or progression to colorectal cancer. Changes in cell-to-cell junction molecules can lead to cellular migration, invasion and metastasis of cancer cells in a tissue-dependent manner. Tight junctions are protein complexes near the apical surface of the epithelial cell and have been found to promote cellular polarity, epithelial barrier maintenance and paracellular transport. Tight junctions are compiled by multiple building blocks, including Claudins, which play a vital role in tight junction anchoring onto...
We extracted total RNA from fresh frozen tissue biopsies of 17 patients (±5 years), sex, race/ethnicity, and colonic segment. Each unrelated control was matched to each case per age, served as the control tissues when the RNA quality of normal adjacent mucosa samples were snap-frozen in liquid nitrogen and stored at −80 °C within 10 minutes from obtainment. The pathological report confirmed the diagnosis of colon adenocarcinoma, and the tumor volume for all was >60%. We defined the stage of cancer using the TNM definition of the American Joint Committee on Cancer. Early-stage colon cancer was defined by no regional lymph node metastasis and no distant metastasis.

In a colonoscopy-based case-control study, we also obtained colon mucosa samples from 5 unrelated individuals who underwent screening colonoscopy and were found to have a normal colon at the Michael E. DeBakey Veterans Affairs Medical Center and Michael E. DeBakey Veterans Affairs Medical Center approved the study protocol.

**Methods**

**Study participants and sample collection**

Our study consisted of 6 patients who were diagnosed with colon cancer and underwent total curative surgical excision at the affiliated hospitals of Baylor College of Medicine between 1999 and 2013. All study subjects had no personal history of malignancy and did not receive preoperative neoadjuvant chemotherapy. The research coordinators collected the tissue samples during the surgical procedure. The resected tumor tissue and normal adjacent mucosa samples were snap-frozen in liquid nitrogen and stored at −80 °C within 10 minutes from obtainment. The pathological report confirmed the diagnosis of colon adenocarcinoma, and the tumor volume for all was >60%. We defined the stage of cancer using the TNM definition of the American Joint Committee on Cancer. Early-stage colon cancer was defined by no regional lymph node metastasis and no distant metastasis.

In a colonoscopy-based case-control study, we also obtained colon mucosa samples from 5 unrelated individuals who underwent screening colonoscopy and were found to have a normal colon at the Michael E. DeBakey Veterans Affairs Medical Center, an affiliated hospital of Baylor College of Medicine. For each, the collected normal biopsy was immediately placed on the dry ice and transferred to a −80 °C freezer within 20 minutes. These samples served as the control tissues when the RNA quality of normal adjacent tissue of 5 cancer patients was not ideal for gene expression analysis. Each unrelated control was matched to each case per age (±5 years), sex, race/ethnicity, and colonic segment.

**RNA extraction and gene expression analysis**

We extracted total RNA from fresh frozen tissue biopsies of 17 samples (6 biopsies from excised cancerous tissues, 6 biopsies from adjacent normal colon mucosa, and 5 from unrelated normal control mucosa) in the genomic core lab at the Texas Digestive Disease Center using the NucleoSpin RNA isolation kit (MACHEREY-NAGEL Inc, Bethlehem, PA, USA). We determined the nucleic acid 260:280 ratio to confirm the RNA purity of samples, which was >2.0 for all. However, the RNA integrity number was <0.6 for 5 normal adjacent mucosa samples. These RNA samples were deemed not adequate for gene expression analysis and thus were excluded from the gene expression experiment. As a result, the gene expression analysis was restricted to 6 resected colon cancer tissues from 6 patients, 1 normal adjacent mucosa from 1 patient, and 5 normal mucosal biopsies from unrelated healthy controls.

We used the Qiagen RT² Profiler PCR Array (Hilden, Germany) to determine the expression of 84 genes in the tight junction pathway. The array includes all claudin genes, and genes encoding OCLN, cell adhesion proteins (ESAM1, ICAM1, ICAM2 and PECAM1), junction adhesion proteins (F11R, IGSF5, JAM2 and alpha actins), catenins (ACTN1-4, CTNNAL-1 and CTNNB1), junction interacting proteins (ACTN1-4, AMOTL1, GGN, YBX3, CTN, EPB41, HCLS1, INADL, MAG1, MAG2, MLLT4, MPDZ, PAR, SYMPK, TJAP1, TJP1-3, VAPA and ZAK), cytoskeleton regulation proteins (AMOTL1, ASH1L, YBX3, CTN, LLGL1, LLGL2, PAR, PAR6B, PARD1, PARD2, SMURF1 and TIAM1), G protein signaling proteins (ARHGEF2, CDC42, CDK4, GNA11, GNA12 and RHOA), and protein kinases (CASK, CSNK2A1, CSNK2A2, CSNK2B, IKK, MAG1, MAG2, MARK2, MPP5, MPP6, PRKCI, PRKCZ and PTEN). The assays were conducted at the core facility at Baylor College of Medicine using the Bio-Rad LightCycler RealTime PCR System (Hercules, CA, USA). After the gene expression analysis, the data from 1 unrelated normal control showed poor signal and we eliminated the data from the analysis. Therefore, we included 6 colon cancer tissue and 5 control tissues in this analysis.

We used the RT² Profiler PCR Array Data Analysis Software, version 3.5, for data analysis. Student’s t-test was used to compare the expression levels between cancer and normal mucosa using normalized gene expression data against ACTB, HPR1 and RPLP0 as the housekeeping genes. We also exploratorily conducted the statistical analysis.
Kourkoumpetis T. et al: Tight junction and colorectal cancer

We calculated fold-change/fold-regulation using the \( \Delta \Delta Ct \) method, in which delta Ct was calculated between the gene of interest and an average of reference housekeeping genes, followed by the \( \Delta \Delta Ct \) calculation: \( \Delta Ct \) (cancer group) − \( \Delta Ct \) (control group). Fold-change was then calculated using the \( 2^{\Delta \Delta Ct} \) formula.

The scatter plot compares the normalized expression of every gene on the array between two groups by plotting them against each other. The central lines indicate the constant gene expression. The dotted lines indicate the selected fold-regulation threshold. Red dots, up-regulated; black dots, unchanged; green dots, down-regulated. The figure was generated from the RT² Profiler PCR Array Data Analysis Software, version 3.5.

### Results

The 6 cancer patients were all non-Hispanic Caucasian men. The age range was 55 to 75 years. Three patients had an early-stage tumor and three patients had a late-stage tumor (Table 1).

When colon cancer was compared with healthy controls, the differential expression of multiple genes was observed (Fig. 1). The \( CLDN1 \) gene was significantly up-regulated, with fold-change of 16 (\( p < 0.001 \)) (Table 2). Four genes were down-regulated after multiple testing adjustments, including \( AMOTL1 \), \( CLDN5 \), \( JAM2 \) and \( TIAM1 \). The differential expression was persistently seen in early-stage as well as late-stage tumors. The down-regulation of \( CTNNA3 \), \( JAM3 \), \( MPP5 \) and \( PTEN \) was not statistically significant after adjustment for multiple testing. \( CLDN2 \) was up-regulated by >25-fold and \( CLDN8 \) was down-regulated >100-fold in tumor tissue compared with control mucosa. However, the difference was

### Table 2. Differential expression of genes in human colon cancer tissue and normal control tissue

<table>
<thead>
<tr>
<th>Gene</th>
<th>All cases versus controls, fold-change</th>
<th>Crude P</th>
<th>Early stage cancer versus controls, fold-change</th>
<th>Crude P</th>
<th>Late stage cancer versus controls, fold-change</th>
<th>Crude P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMOTL1</td>
<td>-3.13</td>
<td>0.007</td>
<td>-2.74</td>
<td>0.07</td>
<td>-3.57</td>
<td>0.03</td>
</tr>
<tr>
<td>CLDN1</td>
<td>16.62</td>
<td>0.008</td>
<td>23.14</td>
<td>&lt;0.0001</td>
<td>11.94</td>
<td>0.10</td>
</tr>
<tr>
<td>CLDN5</td>
<td>-4.89</td>
<td>0.004</td>
<td>-3.63</td>
<td>0.05</td>
<td>-6.60</td>
<td>0.03</td>
</tr>
<tr>
<td>CTNNA3</td>
<td>-3.24</td>
<td>0.010</td>
<td>-3.45</td>
<td>0.03</td>
<td>-3.04</td>
<td>0.09</td>
</tr>
<tr>
<td>JAM2</td>
<td>-7.18</td>
<td>0.002</td>
<td>-5.36</td>
<td>0.03</td>
<td>-9.60</td>
<td>0.02</td>
</tr>
<tr>
<td>JAM3</td>
<td>-2.94</td>
<td>0.012</td>
<td>-2.29</td>
<td>0.11</td>
<td>-3.77</td>
<td>0.04</td>
</tr>
<tr>
<td>MPP5</td>
<td>-2.18</td>
<td>0.015</td>
<td>-2.43</td>
<td>0.08</td>
<td>-1.96</td>
<td>0.08</td>
</tr>
<tr>
<td>PARD6A</td>
<td>1.67</td>
<td>0.092</td>
<td>2.54</td>
<td>0.004</td>
<td>1.09</td>
<td>0.64</td>
</tr>
<tr>
<td>PTEN</td>
<td>-2.36</td>
<td>0.013</td>
<td>-2.75</td>
<td>0.07</td>
<td>-2.02</td>
<td>0.08</td>
</tr>
<tr>
<td>TIAM1</td>
<td>-4.77</td>
<td>0.008</td>
<td>-5.27</td>
<td>0.06</td>
<td>-4.32</td>
<td>0.04</td>
</tr>
<tr>
<td>CGN</td>
<td>-2.09</td>
<td>0.039</td>
<td>-1.04</td>
<td>0.28</td>
<td>-4.19</td>
<td>0.05</td>
</tr>
</tbody>
</table>
not statistically significant (unadjusted \( p > 0.05 \)). Overall, gene signatures and pathway analysis showed down-regulation of tight junction genes in colon cancer compared with normal mucosa (Supplemental Table S1).

We identified three significantly positively enriched Kyoto Encyclopedia of Genes and Genomes pathways (false discovery rate \( p < 0.001 \)), including cell adhesion molecules, leukocyte transendothelial migration and tight junction. Significant contributors to all three sets were members of the claudin family. The primary differentially enriched genes of the tight junction pathway were those related to cell polarity and proliferation and junction assembly (Fig. 2).

**Discussion**

Our pilot findings suggested an altered expression of multiple tight junctions and cell polarity genes in colon cancer compared with normal colonic tissues. Specifically, \textit{AMOTL1, CLDN5, JAM2} and \textit{TIAM1} were the genes significantly down-regulated and \textit{CLDN1} was the only gene significantly up-regulated in colon cancer compared with normal mucosa.

Claudins are a diverse family of transmembrane proteins that aid in the adhesion of tight junction complexes to the intracellular cytoskeleton. At least 24 subtypes have been described, with some of them having a specific tissue predominance and differential expression in specific malignancies. In our study, \textit{CLDN1} was up-regulated in colon cancer compared with normal mucosa. It has been proposed that increased \textit{CLDN1} expression could promote malignant cell behavior via a decrease in E-cadherin by increasing expression of ZEB-1, beta-catenin nuclear translocation and WNT signaling. Our findings were consistent with previous findings in premalignant conditions in humans. Weber \textit{et al.}\cite{Weber} have shown \textit{CLDN1} to be significantly up-regulated in active inflammatory bowel disease, colorectal adenomas and inflammatory bowel disease-associated dysplasia. Bujko \textit{et al.}\cite{Bujko} found increased \textit{CLDN1} expression in 26 samples of colorectal cancer, and 42 adenomas compared to normal colon tissue. In contrast, Ersöz \textit{et al.}\cite{Ersöz} and Suren \textit{et al.}\cite{Suren} have shown that \textit{CLDN1} expression was significantly down-regulated in a series of colon cancer tissues and this lower expression was linked to a higher proportion of lymph node metastasis or aggressive behavior of colorectal carcinoma.\cite{Ersöz,Suren} This difference might be explained by the different gene expression approach used. We evaluated the gene expression at the mRNA levels, whereas Suren \textit{et al.}\cite{Suren} and Matsuoka \textit{et al.}\cite{Matsuoka} used immunohistochemical testing.

We found \textit{CLDN5} was significantly down-regulated and \textit{CLDN2} was insignificantly up-regulated in cancer tissues compared with control mucosa. \textit{CLDN2} is a pore-forming protein and \textit{CLDN5} is a seal-forming protein, and increased expression of \textit{CLDN2} and decreased expression of \textit{CLDN5} are consistent with epithelial dysfunction, as characterized by reduced barrier resistance.\cite{Bujko, Ersöz} \textit{CLDN5} was only shown to be down-regulated in one human study and its role in colorectal tumorigenesis has not been well elucidated.\cite{Ersöz} We did not find significant differential expression of other \textit{CLDN} genes between cancer and normal mucosa, although \textit{CLDN6, 7} and \textit{8} were all non-significantly down-regulated in colon cancer tissue. Nakayama \textit{et al.}\cite{Nakayama} have shown that \textit{CLDN7} expression was significantly down-regulated in human
colon cancer cell lines through hypermethylation of the CLDN7 promoter region. Gene Set Enrichment Analysis revealed a statistically significant difference in the enrichment of these CLDN genes in the Kyoto Encyclopedia of Genes and Genomes tight junction, cell adhesion molecules, and leukocyte transendothelial migration pathways. Further clinical studies are warranted to examine the implication of claudins in colorectal cancer development.

Junctional adhesion molecule proteins are part of the immunglobulin subfamily and involved in the formation of tight junctions in both endothelial and epithelial cells. These transmembrane proteins are believed to aid in cell polarity by anchoring adaptor proteins and tight junctions. We found JAM2 to be down-regulated in colon tumor tissue. Down-regulation of JAM2 has also been observed in two previous studies. Colon cancer cells transfected to overexpress JAM2 were found to have significantly reduced growth, invasion and migration relative to cancer cells with low expression. In contrast, JAM deficiency had a protective effect against melanoma lung metastasis in mice. This is likely due to the role of JAM2 in cell adhesion to endothelium, preventing transendothelial transmigration. In the Gene Set Enrichment Analysis, the leukocyte transendothelial migration pathway was found to be significantly enriched. Analysis of integrated epigenome and transcriptome data identified the epigenetic modulation of cell adhesion molecules in colorectal tumor, with JAM2 being one of four genes down-regulated by hypermethylation. All these studies pinpoint the importance of JAM2 in colorectal carcinogenesis.

The TIAM1 gene encodes a protein that aids in cell anchoring. In contrast to our study that showed TIAM1 was significantly down-regulated in colon cancer tissues, TIAM1 has been shown to be over-expressed in many types of cancers, such as breast and esophageal adenocarcinomas. In oral squamous cell carcinoma, the increased expression of TIAM1 was correlated with lymph node metastasis, earlier recurrence and worse prognosis. One in vivo study found that TIAM1 promotes invasion and metastasis of colorectal cancer, possibly through activation of the Wnt/beta-catenin signaling pathway in a TIAM1 transgenic mouse model. In line with our observation, it has also been shown that suppression of TIAM1 is required for enhanced cell migration and invasion in human colorectal cancer enteroid models.

Our findings also show significant down-regulation of AMOTL1 in colon cancer tissues compared with normal mucosa. This gene controls endothelial cell migration and polarity aiding in cancerous angiogenesis, and its down-regulation has been shown in studies involving cervical cancer and breast cancer. To our knowledge, this is the first evidence that AMOTL1 was down-regulated in colon cancer tissues compared with normal mucosa.

Our study provides novel evidence on the significant differential expression of tight junction genes in human colon cancer compared with normal mucosa. However, several limitations should be noted. First, this was a preliminary retrospective study with small sample size. Thus, false positive and false negative findings could have occurred. The small sample size prohibited the potential for valid subgroup analyses. We also did not collect complete clinical and survival data from our patients and the survival data were not documented for research purposes. This study will be followed by an investigation on the role of tight junction genes on survival of patients with colon cancer in the context of comprehensive clinical metadata and known risk factors for colorectal cancer.

**Future research directions**

We will conduct a large clinical study collecting more comprehensive clinical data on survival of patients with colorectal cancer. If tight junction and cell polarity mechanisms are shown to be causally associated with colorectal cancer survival, dysregulation of tight junctions can be used as potential markers for the prognosis of colorectal cancer. Future studies should also identify microbiome or diet or targeted therapeutic agents to modulate tight junction and cell polarity (such as blocking claudin 1) in pre-malignant or malignant lesions.

**Conclusions**

In conclusion, in colon cancer, the expression of multiple genes related to tight junctions and cell polarity was found to be significantly altered compared with controls. Understanding the molecular mechanism of claudins and tight junction disruption and its clinical significance in epithelial to mesenchymal transition may provide novel insight into colorectal carcinogenesis.

**Acknowledgments**

This research was supported by the Gillson Longenbaugh Foundation and Golfers Against Cancer organization (PI: LJ), Cancer Prevention Research Institute of Texas (RP140767, PI: LJ), National Cancer Institute P30 Cancer Center (P30 CA125123) for support of the Human Tissue Acquisition and Pathology Shared Resources. This project was supported in part by PHS grant P30DK056338 and the expert assistance of Lisa D. White, Ph.D. The research was also supported in part by the Houston VA HSR&D Center for Innovations in Quality, Effectiveness and Safety (CIN13-413).

**Conflict of interest**

The authors have no conflict of interests related to this publication.

**Author contributions**

Study design (LJ), sample acquisition (MI, HBE), data analysis (KER, LC), data interpretation and manuscript writing (TK, KER, MR), critical intellectual input (TK, KER, LC, MR, MI, HBE, LJ).

**Supporting information**

Supplementary material for this article is available at https://doi.org/10.14218/ERHM.2017.00036.

**Supplemental Table S1.** Differential expression of genes in human colon cancer tissue (n = 6) and normal control tissues (n = 5).

**References**


Kourkoumpetis T. et al: Tight junction and colorectal cancer


