The Coupling of the IoT in the Era of Big Data and the CTC Test Detecting miRNA with Using a Molecular Torch Technique

Jiyun Kim
Department of Natural Science, Hankuk Academy of Foreign Studies, Younin-si, Republic of Korea
Email: cathleen0@naver.com

Abstract—**While the incidence rate of pancreatic cancer is low, its mortality rate is still higher than expected. These are due to its recurrent tendency and the difficulty of its early diagnosis. Cancer cell floating around in blood (CTC; Circulating Tumor Cell) is one of the causes of cancer recurrence. On the other hand, CTC shows the specific microRNA (miRNA) unlike normal cells. The miR221 and miR155 are representatives of miRNA in pancreatic cancer. Therefore, the detection of miRNA could be an effective tool for predicting metastasis and recurrence of cancer. MiRNA requires quite sensitive detection methods because of their status as very small amount in blood and its complex structure. This study employs a molecular torch technique for miR221 and miR155. Molecular beacon probe applied to miRNA molecules represents the fluorescence signals. The probe can detect miRNA by even the only one per 10μl both in animal studies and in vitro experiments. For easier and faster diagnosis of cancer it requires supports from doctors and technical equipments. The study constructed and proposed a specific data sharing system to link the probe with an IoT-application applicable to the real life.**

Index Terms—**CTC, miRNA, molecular beacon probes, microfluid PDMS device, IoT**

I. Introduction

A. Study Motivation

The majority of death in the emergency room is due to the existing medical conditions or the recurrence of a previous disease as well as an accident. To reduce the mortality, medical staffs need to give an accurate diagnosis as well as a rapid response to a symptom. The researcher's grandmother once went to an emergency room due to a severe headache, while she was recovering from a spinal surgery. A medical staff in the emergency room ran an electrocardiogram test calmly without panicking and sent the results to her family doctor. The family doctor received the test result through a dedicated application and diagnosed that her myocardial infarction reoccurred. An effective and rapid treatment was possible owing to the service.

As well shown, if a tertiary care institution is far or if the information of a patient is a deficiency, a medical condition of a patient can be rapidly worsened. If either of them is available, many of patients could be treated easily with simple surgeries. If a dedicated application is developed, it will quickly classify and identify required data and it may allow a remote-practice. Through reviewing previous medical records, an appropriate medical treatment can be made promptly without seeking an opinion of a family doctor or an attending physician.

Cancer is the most compelling disease, which requires this kind of apps. Even after cancer is cured, it has a high probability to reoccur due to its high metastatic characteristic. Particularly, pancreatic cancer has a low incident rate but a high likelihood of relapse. Especially, it is anatomically hard to diagnose cancer in its early stage so its relapse has a high mortality rate. If a patient with a history of pancreatic cancer comes to an emergency room, a doctor on duty can consider the relapse of pancreatic cancer if the doctor can review the patient's medical history.

A development of a dedicated application will require cooperation from the maximum number of government agencies and it should be able to provide an appropriate diagnosis by sharing detailed exam records and personal information of a patient. If it merely delivers simple information or the application is malfunctioned, it can cost the life of a patient in a critical condition. It will be needed to validate the needs of its development and ensure the cost of developing the application.

The researcher took following classes to conduct this study. The researcher learned from the lecture of Carol W Greider, a winner of Nobel Prize in Physiology of Medicine, that excessive activation of telomerase prevents the abrasion of telomere to promote the persistent growth of cancer cells. The researcher also could study the diagnosis methods and treatment of cancer including fortifying immune cells by using gene manipulation through a lecture at Seoul National University School of Medicine. Moreover, the researcher studied a cancer stem cell extraction method from the Kyungam Bio Youth Experiment Experience Course held by Korea Society for Molecular and Cancer Biology.

The researcher won a silver medal at the school representative selection competition for the National Youth Science Research Debate Contest by preparing an article of "Analysis on the Information Diffusion Speed

Manuscript received March 31, 2016; revised June 2, 2016.

©2016 Int. J. Pharm. Med. Biol. Sci.

doi: 10.18178/ijpmbs.5.2.99-109

in a Specific Group by Employing a Logistic Model", when the researcher was in 10^{th} grade. The article dealt identified potential services by reviewing the cases of opening and utilizing public data, held by government public sectors, and evaluated if these services can help people in real life. Therefore, the researcher could explore more practical public data usage services. Moreover, the researcher was selected as a scientifically gifted student by Seoul National University School of Medicine, when the researcher was 11^{th} grade, and received mentoring. Upon this experience, the researcher wrote an ARC thesis, entitled "A study on exosome isolation from the blood of a patient with cancer and the molecular beacon for identifying miRNA in tumor cells in the blood". From these two studies, the researcher got an idea of coupling the better utilization of public data and the detection of cancer tumor cell to conduct this study.

The modern society can be called the era of big data. Researchers are actively studying the size of information and the information diffusion rate. The information diffusion rate is proportional to the number of people aware of the information as well as that of people not aware of the information. It is because it considers the potential probability of a person, who does not know the information yet, learn about the information. Determination from a logistic model showed that information diffusion rate is slow at the beginning but it picks up velocity as it spreads. The development of the application and the information sharing can be achieved, only if the public are aware of the efficiency of data sharing through evaluating the possibilities. This can be a means to resolve many issues slowing the first aid response time, such as sending a medical staff or transporting a patient to a hospital via helicopter for people living in an isolated area or an area without a medical facility.

B. The Significance of the Study

In the modern society, cancer has the highest mortality rate than any other diseases. It is because cancer cells tend to evolve with adapting to the environment and complete recovery is impossible, which is a dilemma. The mortality rate of pancreatic cancer is similar to the relapse rate of it. It is because it is hard to diagnose pancreatic cancer from test results and an interview with a patient due to the characteristics of a body structure. Fig. 1 the occurrence of pancreatic cancer increases owing to the westernized diet. It is very important to diagnose and treat cancer in its early stage because it can spread to other organs. Cancer has become a very common disease. It can be found from people living in a rural area as well as in an urban area. In order to deal with it, an emergency response plan, if possible remote diagnosis and treatment as well, should be more activated rather than founding paramedical institutes. However, the capitalism makes the money flow only to urban areas. Of course, even the Seoul metropolitan residents have a high possibility that they cannot receive a proper treatment in an emergency room at night. In particular, the society enters into the super-aged society and senior citizens have a high probability to be beneficiaries of these programs, because they have a high rate of cancer spread and relapse.

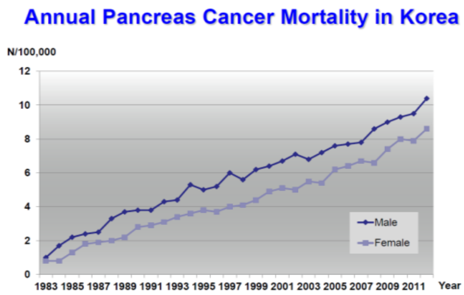


Figure 1. Mortality rate of pancreatic cancer by year

Proper diagnosis and treatment require a method to diagnose a disease quickly as well as an application development. However, the existing cancer diagnosis requires special facilities and medical experts of major hospitals. The society needs to have a diagnostic method, which can identify a spread of cancer or a recurrence of cancer more quickly and accurately.

Circulating Tumor Cell (CTC) cause the relapse and spread of pancreatic cancer but it is impossible to detect it accurately with the current medical technology. It is because a trace amount of CTC is generated. Ref. [1] The miR221 and miR155, existing in the exosome of CTC, are representative miRNA causing pancreatic cancer. It is necessary to introduce a molecular beacon method, which can track the spread and relapse of cancer from trace amount of blood, for detecting it. By coupling microfluidic device with it, researchers can improve the detection probability of the CTC. Although a molecular beacon has higher detection probability, it has a limitation that it only can work at medical institutes specialized for the cancer diagnosis.

Ref. [2] Active data sharing is necessary to overcome this limitation. Information spread through a network very fast. Fig. 2, the information diffusion rate accelerates as can be seen from the comparison of <Kangnam Style> by Psy and a logistic model.

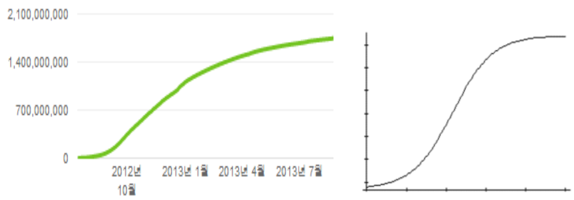


Figure 2. An analysis on the information diffusion rate of <Kangnam Style> by Psy and a logistic model graph

There is a concern that inconsiderate data sharing can leak personal information. To prevent this, a special application is necessary and this application should allow active opinion sharing among medical staffs and smooth communication between medical examination institutes and actual treatment institutes. The information is not simple image or file and it should be applied to treatment equipment. Therefore, it comes with the necessity of IoT (Internet of Things), which can form big data.

C. Study Methods

Ref. [3] The study requires to specialized knowledge on cancer cells and information related to experimental subjects and experimental environment. A researcher must learn how to separate exosome from the blood of a patient with cancer more professionally to detect CTC and determine the detailed miRNA of CTC. Moreover, it is necessary to make the experimental space most efficiently under the given budget and limited laboratory space. A logistic model should be understood to identify big data. Moreover, it is required to measure the increased efficiency when a data web is made in order to convince others regarding the validity of the application development.

Moreover, the researcher requested a mentoring service to the Seoul National University Hospital Biomedical Research Institute to design a system for tracking human organs as well as diagnose cancer. The researcher used the design of the Biomedical Research Institute to realize the molecular beacon method for identifying CTC miRNA and couple microfluidic device. Moreover, the researcher read publications and materials available at the lab, Riss (professional article site), and National Digital Library to acquire information related to CTC (the main cause of pancreatic cancer) and detected miRNA. Moreover, the researcher studied big data from specialized books and related materials and reviewed various cases reported in the news.

The objective of this study was to diagnose pancreatic cancer, which is highly metastatic but impossible to examine visually, by detecting the presence of specific miRNA through CTC test. Pancreatic cancer cells were implanted in an animal model and cancer metastasis was stimulated by induction of inflammation. The researcher wanted to identify the accuracy and speed of an examination by confirming the presence of CTC in blood and tissue. In addition, the researcher wanted to interview a special doctor or conduct a simple experiment to compare the estimated response time and accuracy of the conventional healthcare program or an emergency room system and a case, which information was provided right before the arrival of a patient with using IoT.

II. The Era of Big Data and the Diagnosis of Metastatic Cancer

A. Medical Science in the Era of Big Data

Big Data is a large size data generated under a digital environment, but it has a short generation cycle. It is because of its bilateral data flow characteristics, which make the data fixed and updated bilaterally. Big data mean a large data containing text and video data. The modern society made the internet as a part of life and the size of data has increased heavily. Therefore, it is natural to conduct studies on it. The spread of M2M, exchanging information between a machine and a human or a machine to a machine, is one reason to increase explosively the size of digital information. Moreover, details of human lives are thoroughly stored as data, since all video information (e.g., CCTVs installed on major roads and public buildings) is digitized and stored. Much data are produced at private and public sectors. It is projected that a wide shared network of personal information will be formed in addition to security.

As the name says, big data has diverse and mass data. Therefore, researchers should be able to analyze the data and find meaningful information out of it. It can be useful for the business field, which is supposed to find customers' needs, as well as the medical diagnostic field. In medical emergency condition, a patient goes to a nearby emergency room, not to his or her family doctor. In this case, a doctor on duty has to take a huge responsibility of conducting an emergency treatment with grasping all medical history of the patient promptly.

Doctors often lose the golden time because they have to make a passive approach or wait for a decision. Moreover, they cannot ask questions to an unconscious patient. To prevent this, it is required to have an accurate and fast diagnosis by reviewing the patient's medical history. Moreover, a decision should be made promptly through a quick communication with a family doctor. However, irresponsible personal data sharing can cause various crimes or data collision can mislead improper medical treatment.

Therefore, it is required to identify the diffusion range of medical big data and effective scale of it. It will be widened and the diffusion rate will be accelerated due to the nature of data. A logistic model is a good way to analyze the range of big data. It was originally a method to estimate the growth of a population in an ecosystem. When a logistic model is applied to data, p indicating the degree of saturation of an ecosystem can be used as a proportion of people aware of the information.

$$\frac{dP}{dt} = \mathrm{kp}(1-\mathrm{p})$$

p: the degree of saturation of an ecosystem, $0<p<1$.

There are two requirements to apply this logistic model. The first is the size of the user population is constant. The other is all members of the population are connected, directly or indirectly. These characteristics seem restrictions. It tends to deal with 'data diffusion' and 'data management' at the same time because the data needs to have security measures due to the importance of the data. It is the most suitable to apply it to medical field because the leak of medical information can be wrongfully used by insurance companies or criminals and medical experts may not provide information of patients. Moreover, a patient may deny taking a medical examination or being diagnosed because the patient does not believe doctors. Therefore, as the big data gets larger, the authority to access data should be restricted more. It asks a necessity to create an access authority so only certified doctors could access to the data at specific machines. It also forms a network of doctors to hear an advice of a doctor working for a specialized hospital and one can receive a medical treatment from a family doctor.

Moreover, an issue can occur after receiving the information of a patient. As the work of an emergency room fights for the time, manual input time should be reduced. Therefore, direct data transmission to medical instruments should be allowed by using the IoT.

B. The Molecular Torch Technique to Diagnose Metastatic Cancer

Ref. [4] Metastasis of cancer can be detected by the occurrence and distribution of CTC, which was stated before. CTC is separated from the primary cancer and moves to other tissues or organs through the blood vessels and lymphatic vessels to spread cancer. If the nucleic acid forms of material, which is produced from cancer cells, are detect, it can be used to determine the presence of CTC and diagnose metastatic cancer. However, CTC exists in low frequency (i.e., 1-10/ml) in the blood of a patient with metastatic cancer. Therefore, it requires a technique that can improve the detection rate to obtain accurate results.

Ref. [5] Cancer excessively grows in a limited space and depletes nutrients of the surrounding environment to induce the oxygen deficiency and the accumulation of waste. Fig. 3, as a result, it causes the inflammation of surrounding tissues and makes them cancer cells. Cancer cells are converted from epithelial cells, which remain in a particular space, into mesenchymal cells, which roam freely. Fig. 4, in other words, metastatic cancer means cell movement by this Epithelial-Mesenchymal Transition (EMT) phenomenon and the use of other organs or tissues by (Mesenchymal-Epithelial Transition (MET) phenomenon, which is a reverse of EMT, to divide cells and grow.

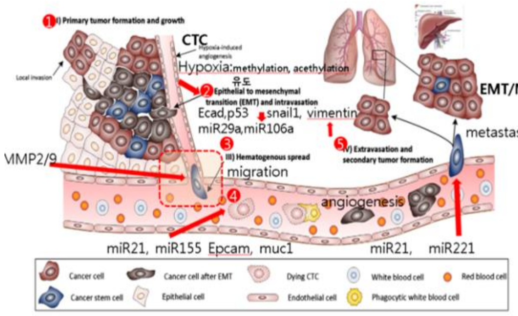


Figure 3. The process of remotely spreading CTC produced from cancer cells

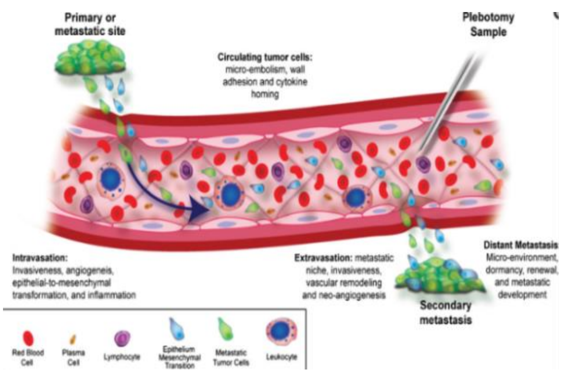


Figure 4. Course of the moving and process of CTC during the spread from the primary cancer

Ref. [6] miRNA is a small micro-substance that was relatively recent found. MiRNA is composed of 20 to 25 nucleotides. It directly influences the function of cells by complementarily binding to miRNA sequence and controlling the transfer. CTC, produced by the miRNA creation, contributes to the spread of cancer. On the aid of phagocytic cells, CTC lyses blood vessels and enters into the blood. This is EMT phenomenon and it gives a condition to invade other organs. Ref. [7] Therefore, miR221 and miR155 are expressed in the CTC. After it is metastases to another organ, it will inhabit and proliferate in the organ through a MET phenomenon. Considering this, CTC is the beginning point of metastatic cancer, so early detection of miRNA of CTC can be an innovative tool to predict and diagnose cancer early. In other words, if miR221 and miR155 are detected from the blood of a patient, researchers can diagnose the metastatic of cancer.

One of effective methods to detect a specific miRNA is the molecular beacon probe method, which is designed by mfold, a program predicting the 2-order structure of RNA. It is more effective than conventional methods. It is a system composed of a hairpin structure containing a complementary base sequence fitting to the target miRNA and a system to predict probe-fluorescent part.

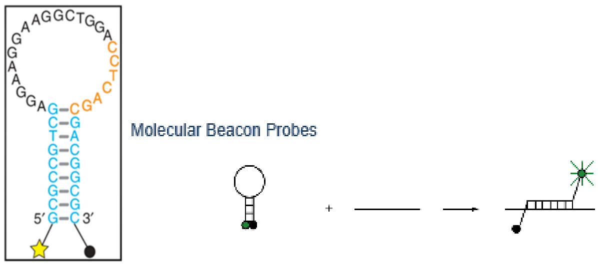


Figure 5. The theory of a molecular beacon probe and the fluorescent observation (molecular torch technique)

Ref. [8] A beacon has non-fluorescent part at one side and fluorescent part at the other side, so it has a property to cancel energy due to proximity. Fig. 5, however, with the presence of target, a beacon couples with miRNA to make non-fluorescent part and fluorescent part apart. The light energy of fluorescent part is displayed on the fluorescent light in the ultraviolet region. Due to this phenomenon, the molecular beacon probe method is called a molecular torch technique.

Ref. [9] Therefore, it is desired to detect nucleotide sequence specific nucleic acid (miRNAs) for each cancer in order to detect CTC from various cancers. Cancer diagnosis can be easier by identifying and classifying nucleic acids. In order to increase the specificity and accuracy of these methods of detection, the microfluidic fluorescence sensor in the spotlight recently can be an alternative option. The microfluidic fluorescence sensor coupled with the beacon method can amplify CTC to enable a quick diagnosis. Moreover, the combination of it with the IoT lowers the barriers of medical examination, which was only possible in the specialized agencies and enables remote medical care for prompt diagnosis and medical treatment. It will minimize mortality and determine the situation of metastatic cancer quicker, since a patient with cancer requires prompt medical attention.

III. PREPARING THE COUPLING OF CTC-MIRNA AND IOT (INTERNET OF THINGS)

A. Pancreatic Cancer Mouse and CTC Analysis

The method to detect CTC-miRNA with using the molecular torches technique is more accurate, effective, and timesaving than other conventional methods. However, the form of molecular beacon probe and medications for the technique are only treated in a special medical institute and the testing equipment is too expensive for general hospitals to equip. Therefore, long distance diagnosis is impossible. A transmission method using IoT, previously described, can be a method to overcome the disadvantage of physical and temporal distance. If general information, a patient’s condition, and sample are transmitted via IoT, it is possible to deal with a situation immediately by using medical tests.

It is required to identify how accurate and fast the CTC-miRNAs test is before promoting such coupling. For this reason, the researcher prepared a mouse with pancreatic cancer as a specimen. Pancreatic cancer is the hardest to diagnose visually so this cancer need the CTC-miRNAs test the most. The researcher wanted to acquire the validity of the CTC-miRNA test, which is the core of this study, through an experiment. A virtual fluorescent probe was prepared and it was applied to the mouse to see if an accurate diagnosis and effective treatment are possible.

1) Study procedure

The goal of this study is to detect miR221 and miR155 among miRNA, which cause pancreatic cancer. An icebox and a professional machine were used to configure a molecular beacon fluorescent probe for detecting them. Moreover, the 8-week-old female mice were used as specimen. It was housed and fed in a single object by weight of 25g at 22 degrees. They were fed at underground first floor A15 room, Bundang Seoul National University Hospital Biomedical Research Institute.

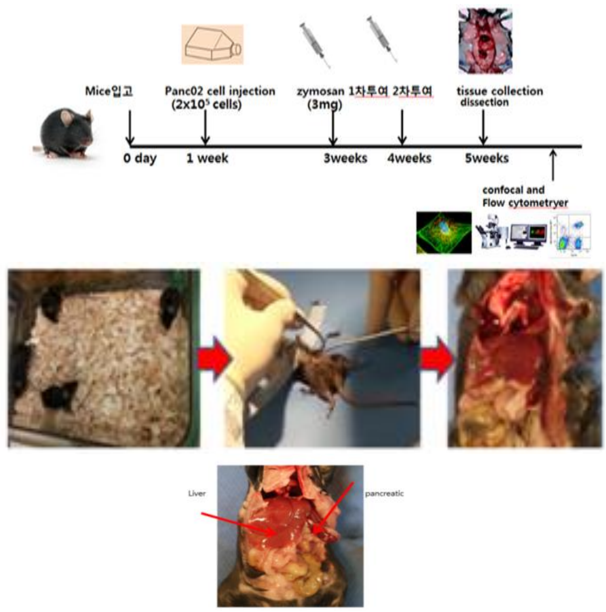


Figure 6. A picture of panc02 pancreatic cancer cell injection to C57BCL mice and transplant procedure

Fig. 6, the procedure of the experiment was as follows. Animal models with pancreatic cancer were created and inflammation was induced. Five C57 Bcl female mice were selected as panc02 pancreatic cancer cell strain model. Then, cancer cells were injected with the diabetes insulin syringe. Inflammation was induced by zymosan after 2 weeks, since inflammation should be caused in a short period.

After manufacturing a fluorescent probe to detect miRNA, the researcher sampled the blood of experiment mice and dyed its tissue. Pancreatic cancer cells in blood were subject to be detected through a CTC analysis.

Mice were acclimated for a week to be adapted to the experimental environment. Abdomen was open under local inhalation anesthesia and prepared pancreatic cancer cell panc02 was injected. The incision was sutured. Mice were fed for 2 weeks with maintaining the body temperature with an infrared lamp. After two weeks, 3mg/100μ of zymosan was injected to the abdomen in order to accelerate the progress of pancreatic cancer state rapidly. It was injected twice with a week apart to induce inflammation.

2) Manufacturing and applying a fluorescence probe

For the fabrication of a molecular beacon probe, fluorescent part and non-fluorescent part were coupled at the 5'-end of a nucleic acid’s base sequence region and at the 3'-end of it, respectively. Then, PCR was conducted for 2 minutes at 94℃ and for 5 minutes at 72℃. Since then, it was cooled for 10 minutes at 4℃ or in an icebox. As a result, it was possible to complete the molecular torch base sequence having a secondary structure.

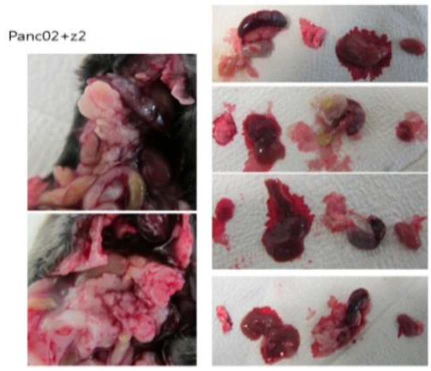


Figure 7. The panc02 pancreatic cell strain of C57BCL mice

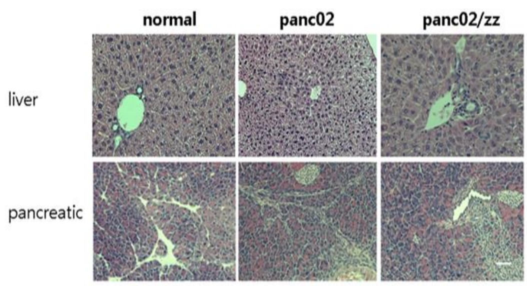


Figure 8. Anatomical opinion of liver and pancreas tissue through H/E stain

After incising the abdominal cavity of a mouse under inhalation anesthesia, the 1ml of blood was sampled from the heart. Fig. 7, the liver and pancreatic tissues were sampled and fixed by immersion in 4% formalin solution. Paraffin-embedded tissue was cut to a thickness of 5µm and fixed to the slide. Fig. 8, then, H / E staining was conducted. This makes it possible to confirm the presence of the miRNA, the target agent at the fluorescent part portion.

3) CTC analysis results in blood

Poly Dimethy Siloxane (PDMS) was used as a material of microfluid device. PDMS is a material proper to manufacture frame for testing small quality of blood accurately and conveniently. It is transparent and non-toxic, so it is good to replace glass slide.

Fig. 9, in order to confirm the presence of CTC in the blood of pancreatic cancer animal model, the 10µ of beacon probe for miR155 and miR221 and 10µl of blood were mixed in PDMS and observed with confocal microscope.

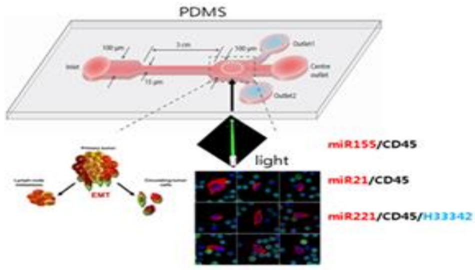


Figure 9. Microfluid fluorescence sensor

4) CTC-specific miRNA molecules image interpretation

Tissues were soaked in formalin solution for 2 days to complete the paraffin-embedded. It was treated in zylene solution for 2 minutes then soaked in 100%, 95%, 85%, and 75% alcohol for 1 minute each. Fig. 10, acquired fluorescence images were analyzed.

A normal mouse had regularly arranged perivascular tissue. A cancer cells injected mouse had slightly deformed cells but did not show much difference with a normal mouse. Two weeks after injecting a medicine activating cancer cells, the symptoms of abnormalities appear around the hepatic portal vein tissue of the treated mouse. The researcher observed that morphology of cells became different with surround cells and abnormal proliferation occurred. The pancreas tissue of a normal mouse did not have cancer cell, but the treated mouse showed as necrosis of cancer tissue as well as rapid necrosis around the vessel.

The researcher observed luminous fluorescence images, by shooting a laser beam on the specimen collected by a microfluidic, with a confocal microscope. MiRNA221 and miR155 are not expressed in a normal group, while miR155 is expressed weakly and MiR221 was hardly expressed in the treated mouse group. For treated mice, inflammation was induced to facilitate metastasis cancer. Fig. 10, as cancer cells proliferated, miR221 and miR155 expressed stronger fluorescent color. Ref. [7] It confirmed that EMT phenomenon was occurring by transforming to CTC due to zymosan.

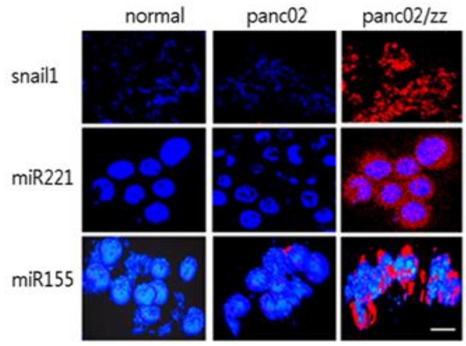


Figure 10. The presence of CTC was confirmed from pancreatic cancer induced animal model mice. Scale bar; 20µm

Ref. [10] To subdivide previous images, Fig. 11, the researcher took 3D pictures of EP-CAM and MUC1, CTC markers, with using confocal and re-divided them into 4D. From the nested images, yellow and blue in red fluorescence EP-CAM and green fluorescent MUC1 images indicate CTC and EMT tissues, respectively.

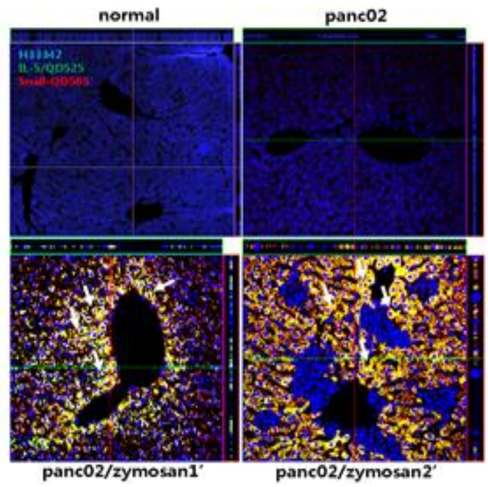


Figure 11. dimensional imaging of liver and pancreas tissue confocal fluorescence

IV. The Era of Big Data-The Efficiency of Medical Treatment with Using IoT

To identify the range of big data and the diffusion velocity, a logistic model formula was introduced. The researcher tries to get the number of people who know the information at time while p means the percentage of people knows the information p. The velocity of information diffusion is proportional to k. The diffusion velocity k is related to the size of a population (L), connection state of a graph, and the unit time of information exchange. The following graph can be estimated from a logistic model formula.

$$\frac{dP}{dt} = \mathrm{kp}(1 - \mathrm{p})$$

As time passes, increment of people knowing information gets larger. Fig. 12, the number of people and the size of population uniformly increase in proportion to the velocity and the expected range of information diffusion at the beginning stage. It picks up speed some time later.

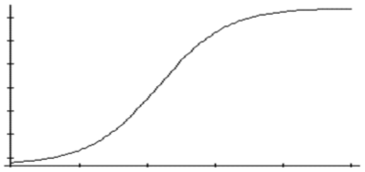


Figure 12. The increase of data sharing speed according to a logistic model formula

A. *Comparing the Response Time of the Conventional ER System and That of the IoT-ER Link System*

The researcher intends to identify how the new system can improve the efficiency of diagnosis and treatment compared to the conventional system, when the IoT-emergency room link system is established based on the diffusion rate estimated from a logistic model. Moreover, the researcher wants to evaluate the merit of IoT link and items to be supplemented. The recent remote medical treatment support system made it possible for medical staffs to perform simple medical treatment and request full diagnosis to a hospital in the distance. However, it requires a patient to come to a medical service. It means that if someone gets hurt or has a seizure in the outdoor, the person cannot receive a medical attention from medical staffs in medical institutes. An application for urgent patients is available now. ‘The emergency patient call service application’ is based on smartphone and it allows voice talk, videotelephony, and sending location information. It can connect the patient to an emergency service by one touch. However, a medical treatment after simple visual inspection cannot be more than superficial treatment. Especially, it has limited application to patients suffering from the relapse of previous diseases. Therefore, only usefulness of the application is to inform the location of a patient more precisely.

In addition, an emergency room strictly applies the triage principle, which means medical attention should be given to patients that are more critical first. The triage’s first order of disease includes myocardial infarction (6-12 minutes), heart attack (4-6 minutes), cerebrovascular disease (3-6 minutes) and severe trauma (within 1 hour). They can be visually identified or diagnosed in an ambulance. However, when it is due to a relapse of previous disease or metastatic cancer, their medical status cannot be assessed visually. When a general patient enters into an emergency room, the examination order will be a nurse, a doctor on duty, resident, and a special doctor at the end.

However, when it is coupled with IoT, a medical staff can receive personal information and previous medical history with conducting simple tests on the patients (e.g., blood sugar), so the severity of the condition can be identified simultaneously. Although blood work results are the same between a normal patient and a patient with metastatic cancer, the meaning of numeric value is different between them. Of course, it can be impossible to use IoT seamlessly and give an accurate diagnosis due to busy movements of an emergency room.

The researcher is aware of the possibility that IoT can exploit the personal information of patients and an instrument error can freeze the whole emergency room if medical staffs highly rely on an instrument rather than try to identify the status of a patient, which can be a disadvantage of this approach. Conducting an experiment in an emergency room violates bioethics. Therefore, the researcher conducted a survey targeting patients with severe illness who visited an emergency room more than 4 times, doctors who worked as emergency room residents, nurse working at an emergency room. Fig. 13, a questionnaire has 12 items and each question is supposed to identify the satisfactory levels (i.e., concerns, dissatisfactory, and satisfactory). The researcher had 56 respondents (15 doctors, 10 nurses, and 31 patients). Each distribution graph focuses on patients willing to use an IoT service and followed by doctors with rich emergency room experiences. Moreover, the researcher sought for questionnaires from nurses who received patients or conducted simple tests.

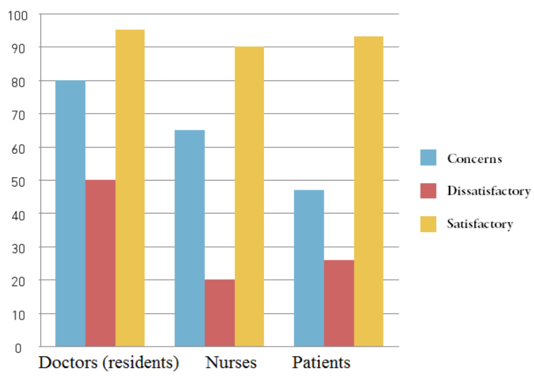


Figure 13. A preference survey on the IoT-emergency room coupling system

Doctors complained about the conventional emergency room system. Moreover, they indicated that the efficiency of coupling would be great because of difficulties in communication with rescue workers in an ambulance and unstable test results. However, some shared concerns on IoT system introduction because of instrumental errors, unstable information, and delay due to differences in data quantity among patients. Moreover, there was a concern that focusing on data may prevent them from examining patients properly because on-site examination should be a priority in an emergency room.

Many patients showed that they often felt discriminated because of triage principle of the conventional emergency room. If a severe disease is overlooked as a mild disease, it can be exacerbated. Therefore, they paid an attention on the merit that medical staffs could review and identify previous medical records and disease symptoms. However, they also worried that personal information can leak to have them disadvantage in insurance or increase the medical bills. Moreover, they concerned that some hospitals may refuse to accept them if they are severely ill, depending on the saturation of an emergency room or the severity of their symptoms.

©2016 Int. J. Pharm. Med. Biol. Sci.

Nurses conduct a brief inspection and manage the patient. Therefore, they must accurately grasp the situation calmly in the emergency room. Majority of nurses were satisfied with the new system because basic test can have lower accuracy in the emergency room situation. However, they concerned about the cases if patients' family rejects using IoT instruments due to personal information leak and increased workload since they have to manage additional IoT equipment.

To reflect these concerns, an emergency room must have an IoT specialized nurse to improve instrument maintenance. Moreover, to prohibit a hospital to refuse accommodating a patient, related regulation should be forced strictly and a communication network should be developed for ambulance rescue workers to transport a patient to a hospital, where the patient can receive an immediate medical attention. In addition, a dedicated application should be created to minimize data collision and encrypt personal information to prevent a leak of patients' personal information.

B. Comparing the Effectiveness of the Conventional Healthcare Program and That of the IoT-Customized Healthcare System

As the interest on health increases in the modern society, healthcare has become an essential welfare, not a choice. It is natural to see the popularity of a healthcare program, considering that companies and public institutes recommend regular health medical checkup. These healthcare programs are considered as a mandatory requirement, not an extra welfare of a company. For example, Tong Yang Investment Bank decided to introduce a healthcare program to celebrate the month of family (based on 2010.05). The purpose of this program is to improve work productivity via health promotion of employees and increase the satisfactory level of employees by advancing work environment. It is composed of smoking, drinking, and overwork categories and it gives each employee a task to check his or her health condition and improve it. If one achieves this task, the company donates a certain amount of money to social organizations in the name of the employee. It means that a company should pay enough attention to employees, since they spend most of their time in the company.

A condominium or a residence complex often designs or offers a healthcare program. For example, it gives a discount on various health examination cost on a contract with a nearby hospital or establish a park for residents to take a walk. Public institutes also send medical staffs to respond or listen to the requests of people in medical needs.

In addition, patients with a particular disease receive recommendation from insurance companies and the Ministry of Health and Welfare. This is mainly because a main disease of the modern society is cancer and cancer requires consistent management, not a cure. However, such healthcare programs focus on superficial health management or overlook some symptoms due to household issues, which are shortfalls. It looks good but it is not practical. To prevent this, one must know their exact health condition.

A healthcare program utilizing IoT should be able to check the blood glucose level and pulse rate more directly than existing smartphone applications. Considering that professional measuring devices are expensive, the functionality of the equipment should be reduced with increasing practicality. Data transmission should be the focus of the instrument. A proper medical treatment can be given upon the diagnosis of a doctor or a medical staff based on the transferred data.

Fig. 14, the questionnaire has 12 items, which were the same as the previous one. It is composed of questions, which allow the researcher estimating the concerns, dissatisfactory, and satisfactory of a respondent. The researcher had 50 respondents (30 patients, 11 healthcare specialists, and 9 ordinary persons). The researcher gave priority to critically ill patients who were going to use the healthcare program the most often. The patients were either suffered from a severe illness or recommended to use a healthcare program or currently experiencing a healthcare program due to lengthy hospitalization. It is hard to define a healthcare specialist, but the researcher asked for a response and an advice to rehabilitation experts who design a rehabilitation program and family doctors. Ordinary people have a low accessibility to a healthcare program because there is a preconception regarding high cost or an insurance problem.

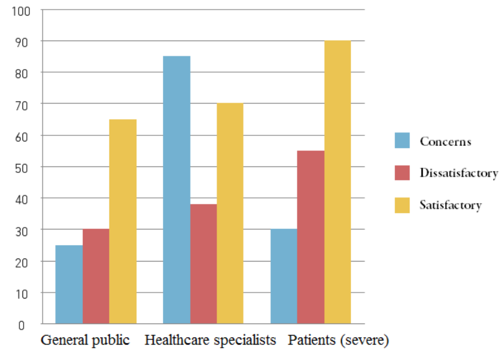


Figure 14. Survey on the preference of IoT-healthcare program coupling

Patients with severe illness showed high satisfaction because they could receive a professional rehabilitation treatment without visiting a medical facility in the long distance. They also expected to reduce healthcare cost because insurance does not support some healthcare programs. Naturally, some concerned about the cost to purchase a special instrument or not meeting a doctor face-to-face. Healthcare specialists were satisfied with the efficiency and the fatigue-reducing effect of patients. However, many of them worried that they cannot directly diagnose the patient's condition and the treatment heavily relies on partial data not by direct physical examination. The public had little feeling of rejection because many of them experienced S health of the current Galaxy, mi band of Xiaomi, and the I-health program of Apple. The existing health exams just offer some discount or restrict the number of testing. Moreover, people cannot take an advantage of it due to their busy daily schedules. They showed high satisfaction because they anticipated that it would be convenient if they could access to special test, diagnosis, and medical staffs through IoT. However, they showed concerns on personal information leak and the negligence of medical staffs.

Two experiments showed a common concern of personal information leakage. However, this can be supplemented with dedicated applications and the data access permission. Furthermore, video chatting with a medical staff will dilute the worry on the lack in face-to-face examination. Saving examination time might be good enough to compensate these shortfalls. In addition, it is advantageous to increase the interest on personal health for people with metastatic cancer as well as the public with the potential of cancer through IoT.

V. The Coupling of CTC-IoT

A. *CTC Preparation for Coupling IoT-CTC*

1) *miRNA preparation in the blood*

Fig. 15, first, miRNA in exosome, secreted from cancer cells, should be detected. To achieve this, the blood cell materials, hindering diagnosis, should be removed efficiently. When blood is centrifuged at the gravity of 1000g for 5-10minutes, the blood cell materials, denser materials, sink in a test tube and form a pellet. The plasma will be on top of the test tube.

Figure 15. Centrifuge (left) and test tube showing separated plasma and blood cell materials (right)

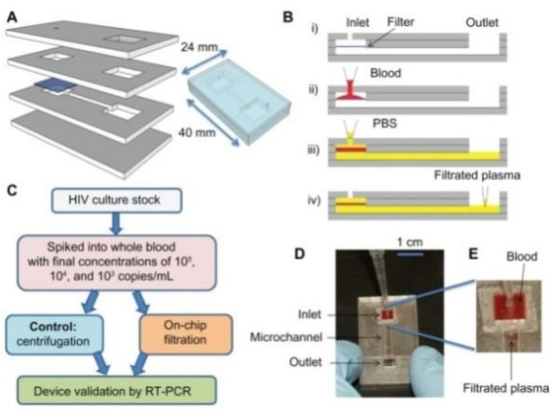


Figure 16. Blood cell material separation process with using microchip (A-D).

Blood cell materials separated by centrifugation can be contaminated by plasma, which is a disadvantage. To overcome this, previous studies were reviewed. Ref. [11] The researcher concluded that filter based microchip method would be suitable for plasma separated, which was introduced at Simple filter microchip for rapid separation of plasma and viruses from whole blood published by Harvard Medical School in 2012. Fig. 16, red blood cells have a diameter of 6.2-8.2 um and a thickness of 2-2.5um. Therefore, it passes through a 2um sieve microchip. Blood cells will be filtered and only plasma will be moved to outlet through a capillary phenomenon.

However, RNA is labile acid compared with DNA. In particular, it is easily attacked by RNase (RNA degrading enzyme). DEPC is a chemical used to extract RNA from the cells and it inhibits the activity of RNase. The researcher could conclude that it is possible to make a combination to maintain pure plasma and RNA in it stably, if the researcher filters sampled blood through a microchip and treat with the DEPC-PBS buffer solution.

2) *Efficiency of the pancreatic cancer diagnosis by using the molecular torch technique*

Fig. 17, the diagnostic procedures for pancreatic cancer using molecular torches, which was designed from this study, are as follows.

i) The process to gain stable RNA from blood: Filter with microchip and apply DEPC-PBS.

ii) Application the blood sample to the microfluidic device (molecular beacons)

iii) Numerical measurement with using a small spectrophotometer

iv) Smartphone: Process measurement and transfer data to the server computer

v) A server computer: Adjust measurement and send a diagnostic result to the smartphone

vi) A cancer specialist: Transfer positive and negative pancreatic cancer diagnosis case to the server.

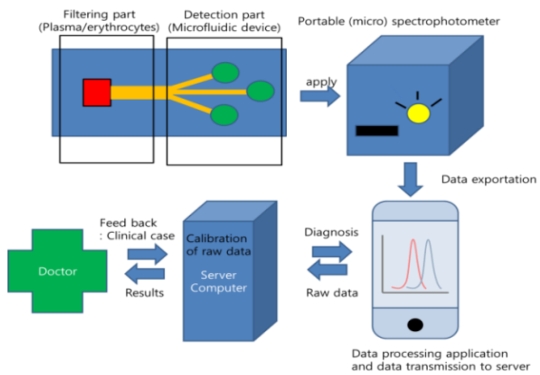


Figure 17. It shows the pancreatic cancer diagnostic fluid device and devices composing it IoT. The flow of diagnostics is also shown.

B. *Preparation of loT for the Coupling of IoT-CTC*

The Device (*Haun et al., 2011*), which was jointed developed by Harvard University and MIT in 2011, is a representative example of on-site cancer diagnosis with utilizing IoT. Currently, the diagnosis of cancer is done by sampling a part of a suspicious tumor tissue. Then, it is sent to a pathology lab to be cultivated. Cancer is determined by observing cancer cell-specific protein or the morphology of the cells. Therefore, it takes a few days for a diagnosis, which is inconvenience. However, this device uses micro NMR (Nuclear Magnetic Resonance) and magnetic nano-particle binding to a cancer cell-specific protein. Fig. 18, the diagnosis of cancer is made within one hour by using a smart phone and a dedicated application to calculate detection results.

Figure 18. Smartphone with application (left) and micro NMR scanner (right) to diagnose cancer

The researcher comes to an conclusion that to make the molecular torch technique, used for examining pancreatic cancer, more efficiently used; i) a semi-expert can easily sample blood and apply it to a microfluidic device; ii) a molecular torch detects cancer inside of a microfluidic device; and iii) data should be transferred to the server via a smartphone or an application in a smartphone can make a final diagnosis. Therefore, the researcher configured reagent, tools, and devices optimized for each step.

1) A fluid device scanner required for the diagnosis of cancer

After coupling pancreatic cancer marker miRNA present in the blood and a beacon complementarily, a device is needed to produce a visible light signal by applying energy to miRNA-beacon compound. Therefore, the device should have i) a lamp emitting UV and ii) a detection with filters to read red and green wavelength. Fig. 19, a spectrophotometer is a device to measure the quantity of these various wavelengths for a long time. It is a basic analytical device used in almost all study fields including chemistry, life science, and pharmaceuticals.

Figure 19. Spectrophotometer

Fig. 20, this product, called a Spectra Academy, is miniaturized by omitting the monitor or the operation unit, unlike the spectrophotometer. The nano-fluid device design has a merit of being thin and small. It will be possible to get detection data regardless of testing location by coupling the proposed technology with this small spectrophotometer.

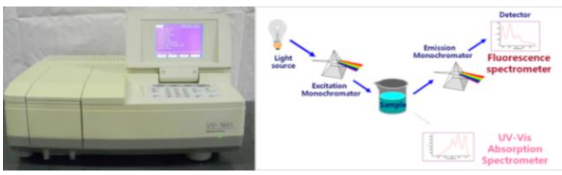


Figure 20. Spectra academy of K-MAC

2) Interlock a smartphone and a server to calculate and interpret the detection result

A smartphone can easily interpret a result, if it has application receiving data from a small spectrophotometer and showing experimental results by tables and graphs. However, it is very dangerous of non-medical experts to determine the metastasis of cancer by simply replying on data given by an application. In order to avoid these risks, the researcher chose a method to deliver measurement to a server and let a server calculate it. The calculated results are transferred to a cancer expert or examiner. Fig. 21, as more people and organizations use this device, the data accumulated in the server increases. Later, a range of positive and negative results to determine the pancreatic cancer will be more clearly displayed. Moreover, it will be possible to adjust various errors, which may influence the measurement, and it will increase the reliability of the test result.

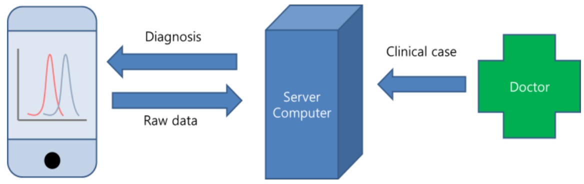


Figure 21. A procedure to diagnose cancer with applying IoT and big data establishment

VI. Conclusion

The objectives of this study were to accurately diagnose metastatic pancreatic cancer using molecular torch technique and develop a dedicated application to accelerate the diagnosis and treatment of the cancer by coupling with IoT. The researcher wants to start a discussion based on the big data theory, a major issue of the modern society, and a logistic model to acquire application development budget and prove the validity of information sharing.

The study conducted two experiments. The first was to confirm the validity of a CTC test with using a molecular torch technique for identifying the spread of pancreatic cancer. The second was to compare the competitiveness of IoT to the conventional emergency room system and the existing healthcare program. The first experiment was conducted under an assumption that miR221 and miR155, which are the causal factors of pancreatic cancer, are effective in determining cancer spread. It is hard to diagnose pancreatic cancer in early stage and relapse of it. Therefore, it has high mortality rate. It is said that genetic manipulation is effective to enhance immune and cure cancer. However, it takes a long time, so faster cure is recommended. Fast treatment is possible only when researchers understand the formation and the recurrence of cancer through tracking cancer stem cell exosome and measuring the size of molecular cell. Therefore, researchers need to find out the type of cancer by detecting specific miRNA expressing cancer cell CTC.

The researcher selected a molecular beacons method for detecting CTC-specific miRNA and tried to couple it with nano fluidic device to enhance accuracy. The researcher manufactured a fluorescence probe model inducing a molecular torch technique. The researcher also confirmed how accurate it is in detecting miRNA, which was produced a little, by creating a mouse with pancreatic cancer. It was concluded from the experiment that it is possible to quickly diagnose the relapse of pancreatic cancer, spread, and a metastasis organ.

The researcher found a method to find the spread of cancer and metastatic cancer accurately and quickly. By coupling this with IoT, the researcher tried to develop a dedicated application, which can diagnose cancer and allow prompt emergency treatment. The era of big data can allow accurate and fast diagnosis wherever a patient is and what circumstance a patient is under, if data of patients can be shared. Even a hospital without a pathology lab to diagnose cancer can diagnose a cancer with a dedicated application, if it has a microfluidic device, portable spectrophotometer, and a smartphone capable of IoT or a simple medical instrument, which can receive data with IoT. If researchers want to improve the accuracy of diagnosis, it will be required to store various data on a server. As more results are accumulated, detection readings will be adjusted to make reading easier and more accurate. Moreover, as data of patients systematically collected and organized, researchers will be able to produce a synergistic effect in all areas related to cancer treatment, such as clinical, diagnostic, and the inspection areas. Of course, it will require the agreement of patients and cooperation of doctors. The last chapter planed concrete application development plan and the composition of IoT system. The researcher listed required instruments for the system and additional required functions were mentioned as well.

In the second experiment, the researcher collected opinions on if IoT was introduced to an emergency room system and to a healthcare program by using a questionnaire. Patients worried of personal information leakage. Medical doctors and healthcare experts concerned about data collision and nurses and ordinary people expressed concerns on instrument management. However, people overall predict that it will provide more accurate and specialized diagnosis and treatment.

References

[1] J. S. Yoo, "Transcriptome-Wide analysis of interplay between ago-microRNA and nonsense mediated rnRNA decay," Master thesis, Graduate School, Sungkyunkwan University, 2015.

[2] J. S. Kim, U. J. Kwon, and T. M. Song, "'Big data' supply and demand prediction by applying social big data: Application of data mining decision tree," *The Korea Society of Management Information Systems*, vol. 2014, no. 1, 2014.

[3] S. S. Choi, S. Y. Chi, K. H. Yoo, and J. K. Kim, "Visualiztion of big data analysis results connecting to database," *Korea Society of Big Data Service*, vol. 2, no. 1, 2015.

[4] Y. J. Chung and H. L. Chong, "miRNA155 inhibitory effect on the proliferation and invasion of salivary gland tumor cell line," *Korean Journal of Oral and Maxillofacial Patholololgy*, vol. 38, no. 6, pp. 5145-5151, 2014.

[5] Z. Bai, J. Sun, X. Wang, H. Wang, H. Pei, and Z. Zhang, "MicroRNA-153 is a prognostic marker and inhibits cell migration and invasion by targeting SNAII in human pancreatic ductal adenocarcinoma," *Oncol Rep.*, vol. 34, pp. 595-602, 2015.

[6] Z. Y. Zhang, Z. L. Dai, X. W. Yin, S. H. Li, S. P. Li, and H. Y. Ge, "Meta-analysis shows that circulating tumor cells including circulating microRNAs are useful to predict the survival of patients and gastric cancer," *BMC Cancer*, vol. 21, p. 773, 2014.

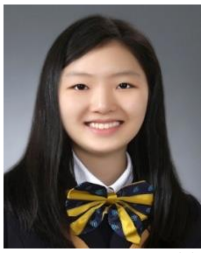
[7] M. Escamilla-Tilch, *et al.*, "The interplay between pathogen-associated and danger-associated molecular patterns: an inflammatory code in cancer?" *Immunol. Cell Biol.*, vol. 91, pp. 601-610, 2013.

[8] S. Tyagi and F. R. Kramer, "Molecular beacons: Probes that fluoresce upon hybridization," *Nat Biltechnol*, vol. 14, pp. 303-308, 1996.

[9] A. J. Armstrong, *et al.*, "Circluating tumor cells from patients with advanced prostate and breast cancer display both epithelial and mesenchymalmarkers," *Mol Cancer Res.*, vol. 9, pp. 997-1007, 2011.

[10] A. M. Fahmy and K. D. Melinda, "Vintegrins and TGFβ-induced EMT: A circle of regulation," *J. Cell Mol. Med.*, vol. 16, pp. 445-455, 2012.

[11] S. Q. Wang, *et al.*, "Simple filter microchip for rapid separation of plasma and viruses from whole blood," DOAJ, 2012.



Jiyun Kim was born on January 8, 1998 in Busan, South Korea. In 2003 she went to Michgan, United States to study abord and was given early admission to 1st grader in Angel elementary school. She came back to South Korea 2 years later. In 2011 she was admitted to Chungshim International Academy (middle school) to be taught only in English in Gapyung, South Korea. Now she is studying in Natural Science Department, Hankuk Academy of foreign studies in Yongin, South Korea.