九州大学大学院 医学研究院

基礎放射線医学分野

Department of Medical Biophysics and Radiation Biology, Faculty of Medical Sciences, Kyushu University

責任者：助教　大野 みずき

(e-mail: mizuki.ohno.700@m.kyushu-u.ac.jp)

場所：総合研究棟７階

TEL 092-642-6143

研究テーマ

DNA損傷とその修復システムの分子メカニズムの解明

放射線や酸化ストレス、あるいは環境中の様々な化学物質によって私たちの細胞のDNAには日々膨大な数の「傷」が発生しています。DNAに生じた「傷」は突然変異や細胞死を引き起こし、がんや老化の原因となります。「DNA修復機構」はそのような「傷」を素早く見つけ出し、修復し、細胞の正常な機能を維持するために重要な役割を果たしています。

私たちは、DNA傷害の発生原因とDNA修復機構に注目して研究を行うことで、生物機能の最も基本的で重要な「遺伝情報の安定維持機構」を深く理解したいと考えています。私たちの研究の成果は、がんや遺伝病の発生原因の解明だけでなく、老化や寿命、更には生物進化の法則を理解することにもつながります。

主な研究プロジェクト

- 環境ストレス感受性マウスを用いた生殖細胞ゲノム変異の研究
- ゲノム損傷応答の不全による突然変異と発がんに関する研究
- 放射線の外部被ばく・内部被ばくの生体影響の研究
- 卵子が持つ精子DNA損傷を修復する能力の分子遺伝学的研究
- 心臓アンチエイジング：ヒトiPS細胞由来老化心筋モデルの構築
- 遺伝リテラシー向上のためのSTEAM教育プログラム開発

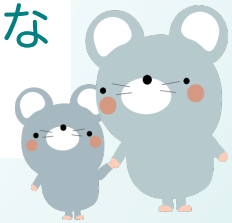
当研究室では、遺伝情報維持の分子機構に関与する遺伝子を人為的に欠損させたマウスや、培養細胞（ヒトやマウス由来のiPS細胞、がん細胞株、初代培養細胞など）を用いて、突然変異、細胞死、発がん、老化の抑制、並びに生殖細胞系列におけるゲノム情報の維持に関する分子機構を明らかにすることを目指した複数の研究プロジェクトを展開しています。

研究プロジェクト1 概要

環境ストレス感受性マウスを用いた生殖細胞ゲノム変異の解析

　体細胞ゲノムに生じる変異はその個体の健康に影響しますが、生殖細胞ゲノムに発生する変異は子供や後の世代の人類にまでも影響する可能性があります。近年の原子力発電所の事故や宇宙での有人飛行の長期化に伴い放射線の人体影響、特に継世代影響の解析の必要性が高まっています。

　このプロジェクトでは生殖細胞ゲノムの安定維持機構の解明のために、DNA修復遺伝子を欠損させたマウスの親子を用いて、一世代で新たに発生した生殖細胞ゲノム変異を次世代シーケンサーを用いて解析を行っています。特に親マウスが放射線被ばくをはじめとする様々な環境ストレスを受けた時の子供のゲノムへの影響などを解析しています。これらの研究結果は「遺伝的変異の発生と抑制の分子メカニズム」の理解につながる重要な知見となります。



研究プロジェクト1 資料

私たちの研究プロジェクトが紹介された記事

Impact Objectives

- Clarify the causes of spontaneous germline mutations and the mechanisms of mutagenesis in mammals
- Detect and analyse *de novo* germline mutations experimentally using DNA repair enzyme-knock out mice

Is genome mutation driving our continued evolution?

Dr Mizuki Ohno, Dr Kunihiko Sakumi and their team from Kyushu University, Japan are investigating the origin of de novo germline mutations in mammals in an effort to better understand how we are likely to evolve


Dr Mizuki Ohno


Dr Kunihiko Sakumi


Dr Noriko Takano

What are germline mutations and what is their significance?

A certain degree of mutation spontaneously arises and accumulates in both somatic and germline cells in mammals. Accumulated mutations in somatic cells increase the risk of cancer and other diseases. Only the mutations that occur in the germline cells can be transmitted to the offspring and thus to later generations.

Germline mutations are the root source of genetic variation and are regarded as a driving force for genome evolution in multicellular, sexually-reproducing organisms like mammals. The rate of germline mutation is an important determinant of evolutionary speed and maintaining the integrity of germline genomes might provide evolutionary advantages.

What have the main challenges been in achieving your goals?

Our main challenges have been the experimental identification of true *de novo* germline mutations and determining the mutation rate to pinpoint the causes and mechanisms of these mutations in mammals. Previous attempts to estimate the germline mutation rate in mammalian species include biochemical methods, phylogenetic methods and monitoring spontaneously arising mutant phenotypes in large populations of laboratory animals. Recent progress in next generation sequencing (NGS) technology has enabled us to detect *de novo* germline mutations directly by comparison of genomic sequences of parents and offspring. However, *de novo* germline mutation rates in wild-type animals are too low to handle experimentally, so we used DNA repair-deficient mice to accurately detect mutations from NGS data.

Have there been any surprising results so far?

The first big surprise was that the oxidative DNA repair-deficient mouse line showed a mutator phenotype. Some abnormal phenotypes attributed to the *de novo* germline mutations were observed in this mouse line because we had not known any other mutator mouse line at that time. The DNA repair genes disrupted in our mice were originally found in a study of *E. coli* mutator strains and it was later found that those are conserved in a wide variety of organisms, including human and mice. That *E. coli* mutator genes might contribute towards controlling germ cell genome integrity was another big surprise.

Oxidative DNA repair-deficient mice have a germline mutation rate approximately 20 times higher than wild-type mice. In other words, these mice are evolving 20 times more quickly than wild-type mice. The mutations for 20 generations in the wild-type mice are acquired in just one generation of oxidative DNA repair-deficient mice. The biggest surprise we found was that significant amounts of the oxidative DNA damage are endogenously generated, then removed and repaired to maintain germ cell genome.

What is the most important challenge to overcome moving forward?

We have several variable mouse strains with deficient DNA repair enzymes or DNA damage response genes for the germline mutation and the somatic mutation studies. Tissue samples of those mice are available. However, the cost of NGS is still too high to sequence all of those samples. We would like to have some support or collaborators and need to have both hard and soft bioinformatics. We would also like to use human germline mutation data because there is more data available than for the genomes of other animals.

What have you learnt from these challenges and how will you implement that learning moving forward?

We can effectively identify base substitution mutations at well-sequenced reference regions from our NGS data, but it is still difficult to identify some mutations found at or near repeat sequences, especially for insertion/deletion mutations. Like most researchers, we have omitted the unconfirmed data from the analysis, so we have possibly underestimated the mutation rate. To overcome these problems, we need to use newer sequencing technology such as long read and single cell sequencing.

The role of germline mutations in genome modification

The human genome has undergone significant change via genetic damage and mutation and it continues to evolve. The mouse de novo germline mutation project seeks to establish how these mutations affect and modify the mammalian genome and determine its future

The significance of the analysis of the fruit fly genome, completed in 1911 by Alfred Sturtevant, has been compared to that of the Wright brothers' first flight at Kitty Hawk. The human genome decoding that was finally completed in 2003 has been a further major step in the fight against an array of inherited disorders, giving us another tool in the drive for personalised treatments. The importance of this step can be extended, in the aircraft analogy, to the moon landing. Understanding the genome and how it drives our physiology represents critical progress in tackling many diseases.

However, analysis of different iterations of the stored genome show that it isn't static and indeed, appears to be in a state of flux, driven by mutations within the system. Our genome has undergone a series of changes over time, instigated by an accumulation of mutations that have arisen since the germ cell genome of our ancestors. While this is fairly well understood, the research being undertaken by Dr Mizuki Ohno is aimed at further uncovering how mutation may affect our genome and where it is likely to lead us on a genetic level as it continues to modify over time. Ohno's team seek to determine the causes for these mutations as well as to identify the contributing factors that influence the mutation rate in mammals.

GENETIC MUTATION: THE PATHWAY TO CHANGE

Gene mutations are permanent alterations in sections of DNA sequences called genes. This causes a significant and distinguishable change in the base sequence of the affected DNA. They are changes to the base sequence that can occur spontaneously or in response to cellular damage and can vary greatly in size and position, ranging from a single base pair mutation, to changes that span segments of chromosomes, across several genes. Mutations in somatic (non-reproductive) cells are not passed on to the next generation, however germline mutations can occur in germ line cells that can produce egg and sperm, thus causing changes to the basic genome to become fixed in the DNA for future generations to come. It is these germline mutations that are of particular interest to Ohno and her team.

While DNA repair mechanisms exist naturally in any organism, they have failed in the case of mutations. Ohno's study delves, not only into how often and what type of DNA damage occurs in germline and somatic cells, but also which repair pathway is important in the correction of germline mutations.

CLARIFYING THE CAUSES OF GERMLINE MUTATION

Every cell in the body relies on the action of thousands of proteins working together in concert to function properly. However, gene mutations can affect this process, preventing one or more of these proteins from acting correctly. Changes in a gene's sequence can alter the protein, causing it to malfunction or even fail to be produced. When mutations affect vital proteins, this can disrupt normal development or cause disease. Thus, where a condition is caused by genetic mutations, they are known as 'genetic disorders' and these have been difficult to predict.

Many of these modifications - so-called *de novo* mutations - are genetic alterations present for the first time in one family member as a result of a variation or mutation in a germ cell in either an egg or sperm from one of the parents. DNA repair systems allow for many of the mutations to be repaired and, in reality, only a low level of them are carried forward in the genome. Ohno and her team are investigating the causes of germline mutation and strive to understand the implications of mutation, with a view to establishing how they may evolve and the possibilities for our future selves. The current human genome is a result of amassed mutations that have accumulated in our genome and driven it along certain pathways to yield what we are now. Ohno is currently working with gene-modified mice but the work is transferable to any mammalian genome, including humans, to determine a possible future pathway.

GERMLINE MODIFICATION AS A DRIVER FOR EVOLUTION

Working with a team including Dr Kunihiko Sakumi and Dr Teruhisa Tsuzuki from Kyushu University, as well as contributors from the Nagahama Institute of Bio-Science and Technology and RIKEN BioResource Center, Ohno has been working to establish the causes of germline mutations and the mechanisms of mutagenesis in mammals. With previous experience in studying oxidative damage to DNA and how dysfunction in the DNA repair pathways may be an initiator for tumour growth, Ohno is well-placed to carry out this type of research. She has previously completed a detailed investigation into lesions – quantifiable damage to base pairings or structure – in mouse DNA and the resulting *de novo* germline mutations. The findings of that study have supported the notion that spontaneous base damage such as 8-oxoguanine, that are generated in the DNA due to the presence of reactive oxygen species (ROS), are responsible for many germline mutations and that the mutations themselves have contributed to our continued evolution.

> *Germline mutations are the root source of genetic variations and are regarded as a driving force of genome evolution in multicellular, sexually-reproducing organisms such as mammals. The rate of germline mutation is an important determinant of the evolutionary speed and maintaining the integrity of germline genome might provide evolutionary advantages*

This is a startling observation and one that has huge implications for the way in which we deal with cellular material. Sakumi says, "We also need to pay attention to how much DNA damage has accumulated in the frozen sperm, eggs and embryos used in human reproductive medicine. From the first test tube baby born in a British hospital in 1978, IVF and related technology has increased the number of people using *in vitro* fertilisation and frozen embryo transfer. If this kind of handling is promoting genome damage and subsequent mutation, then it becomes difficult to predict which changes could become entrenched in our DNA and therefore carried through to future generations.

TRANSITIONING MOUSE GERMLINE MUTATION TO THE HUMAN GENOME

Ohno and her team are currently amassing the data from DNA repair-deficient strains of mouse response genes and while they exhibit a germline mutation rate of up to 20 times higher than that in unmodified mice, translation of the results to both these and the human genome will take much more work. The cost of NGS is prohibitively expensive when examining the huge number of samples and Ohno is actively seeking further support and collaboration to assist with this crucial investigation.

Ohno realises that while the project can reliably demonstrate the mutation device in modified mice, there is still much work required to show that the same mechanisms are occurring in much larger mammals with a healthy DNA repair system in place. If they can reliably prove that connection, the ultimate goal of predicting a future state for the human genome won't be far behind and that opens the door for bespoke and personalised treatments for a number of genetic illnesses and diseases.

Project Insights

FUNDING
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RESEARCH TEAM MEMBERS
- Associate Professor Kunihiko Sakumi, Professor Yusaku Nakabeppu, Medical Institute of Bioregulation, Kyushu University
- Dr Noriko Takano, Associate Professor Yoshimichi Nakatsu and Professor Teruhisa Tsuzuki*, Department of Medical Biophysics and Radiation Biology, Faculty of Medical Science, Kyushu University (* currently affiliated with Fukuoka Dental College)

COLLABORATORS
- Dr Yuki Iwasaki and Dr Toshimichi Ikemura, Nagahama Institute of Bio-Science and Technology
- Dr Ryutaro Fukumura and Dr Yoichi Gondo, RIKEN BioResource Center
- Dr Arikuni Uchimura, Radiation Effects Research Foundation

CONTACT
Dr Mizuki Ohno
Project Coordinator

T: +81 92642 6143
E: mohno@biophys.med.kyushu-u.ac.jp
W: https://biophys.wp.med.kyushu-u.ac.jp/

PROJECT COORDINATOR BIO
Dr Mizuki Ohno is an assistant professor at the Department of Medical Biophysics and Radiation Biology, Faculty of Medical Science, Kyushu University, Japan. She specialises in molecular genetics, cytogenetics, molecular evolution, DNA damage and DNA repair.

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科研費 KAKENHI

研究プロジェクト2 概要

ゲノム損傷応答の不全による突然変異と発がんに関する研究

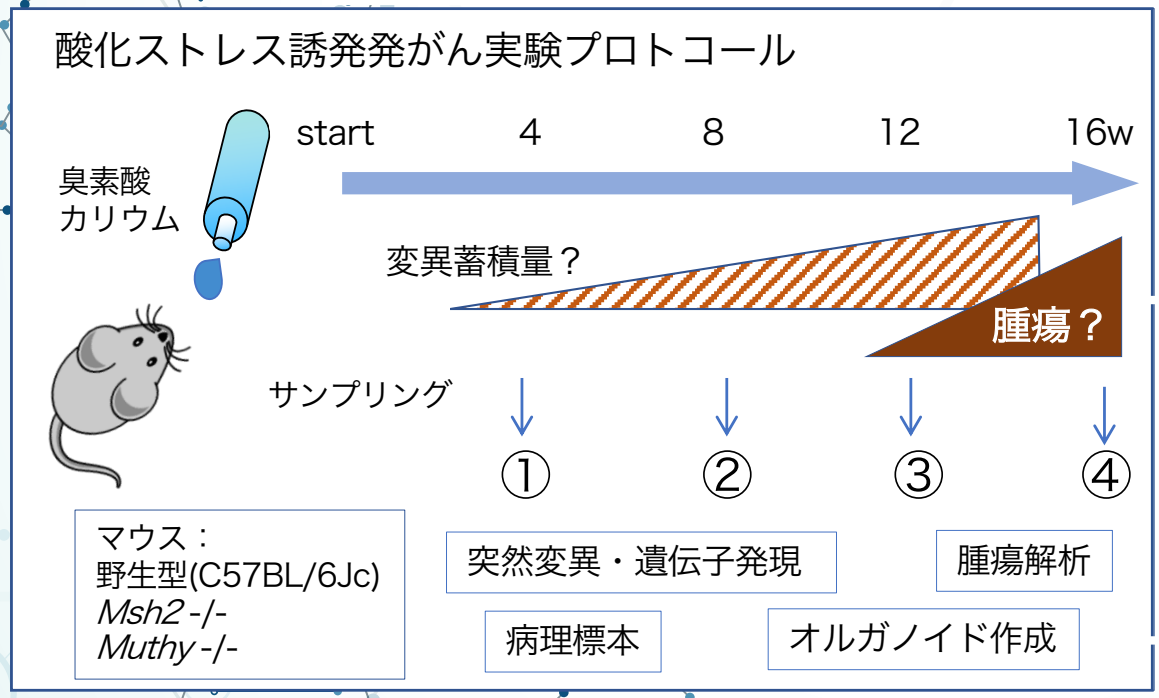
　このプロジェクトでは、ヒトの遺伝性大腸がんのモデルとして、*Mutyh* 遺伝子、*Msh2* 遺伝子などを欠損させた遺伝子改変マウスを用いて個体レベルでの発がん解析を行っています。これまでに、これらのマウスを長期間飼育すると種々の臓器でがんが自然発生すること、また、酸化剤を含む水を継続的に与えて飼育すると短期間で消化管に多数の腫瘍が発生することを明らかにしてきました。これはDNA修復機構が機能しないことで、酸化ストレスによって誘発されたDNA損傷が修復できず、その結果突然変異が過剰に蓄積し、発がんに繋がることを意味しています。

　現在、がんゲノム変異解析やがんのイニシエーションから悪性化までのプロセスに影響する因子の探索を行っています。

研究プロジェクト1 資料

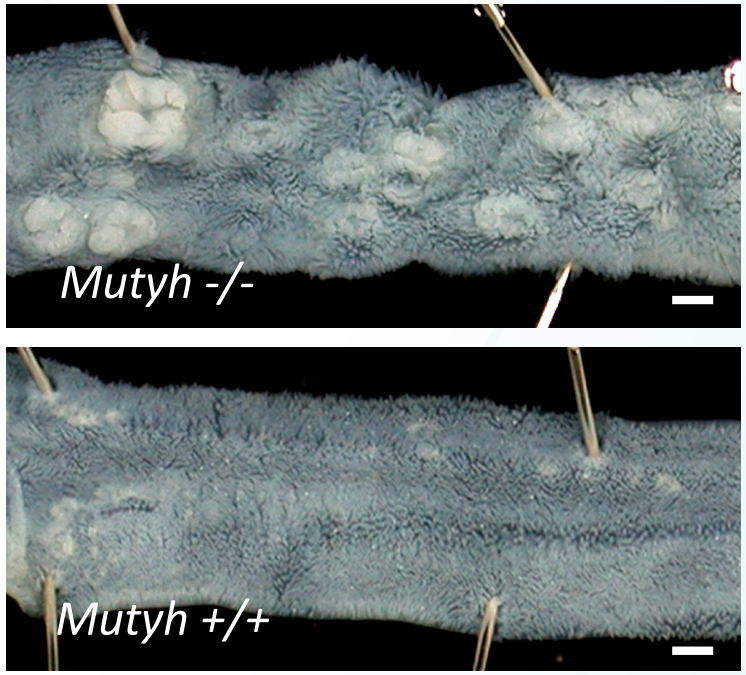
ゲノム損傷応答の不全による突然変異と発がんに関する研究

発がん実験の概要



臭素酸カリウムは食品添加物にも使用されている酸化剤

小腸に発生した腫瘍



参考資料

DNA 損傷は常に発生している

私たちの細胞のDNAには毎日膨大な数の「傷」が自然に発生しています。しかしDNA修復タンパク質がそれらの傷を素早く見つけ出し正確に修復することで「遺伝情報」が守られています。

Spontaneous chemical decay of DNA

Type of DNA damage	/ cell / day
Hydrolysis	**~10,000**
AP sites	
deamination	
Oxidation	**~3,000**
8-oxoG	
thymine glycol	
Methylation	**~4,000**
7-MeG, 3-MeA	
O^6-MeG	
Strand breaks	**~50,000**
SSBs, DSBs	
Total	**≈ 67,000**

Preston BD.et. al. Seminars in Cancer Biology (2010) 20 281–293

Total DNA damaging events

>60,000 / cell / day

↓

6 x10^{13} cells in a human body

~10^{18} DNA damaging events / person / day !!!

↓

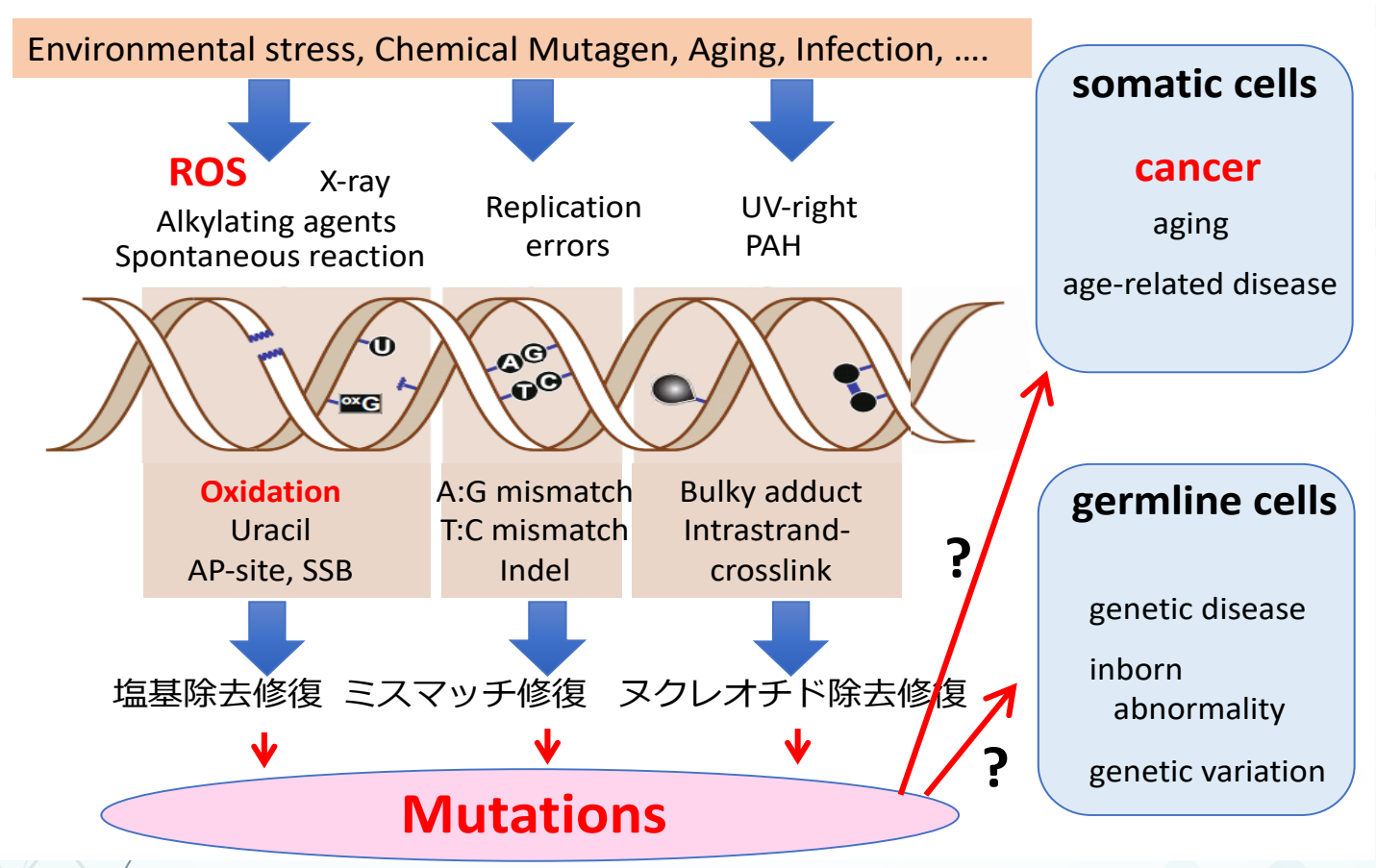
>10^{16} cells are produced over a life time

How many DNA damages must be repaired ???

参考資料

DNA 損傷の種類とDNA修復の種類

様々な原因で異なる種類のDNA損傷が生じ、それぞれに特異的なDNA修復機構が働きます。修復機構の不全や修復容量以上の損傷が生じた場合は突然変異が引き起こされ、体細胞ではがんや老化を誘発し、生殖細胞では遺伝病、不妊、流産などの誘発要因となります。



研究室メンバー

助教　大野 みずき

(e-mail: mizuki.ohno.700@m.kyushu-u.ac.jp)

共同研究員　日髙 京子
岡素 雅子
鷹野 典子
盛 子敬

テクニカルスタッフ　佐々木 史子
石原 邦子

ご質問、お問い合わせはメールまたは電話で

基礎放射線医学分野

TEL 092-642-6143