Summary of the proposed research

Early investigation into the mechanisms leading to tumor dormancy in lung cancer

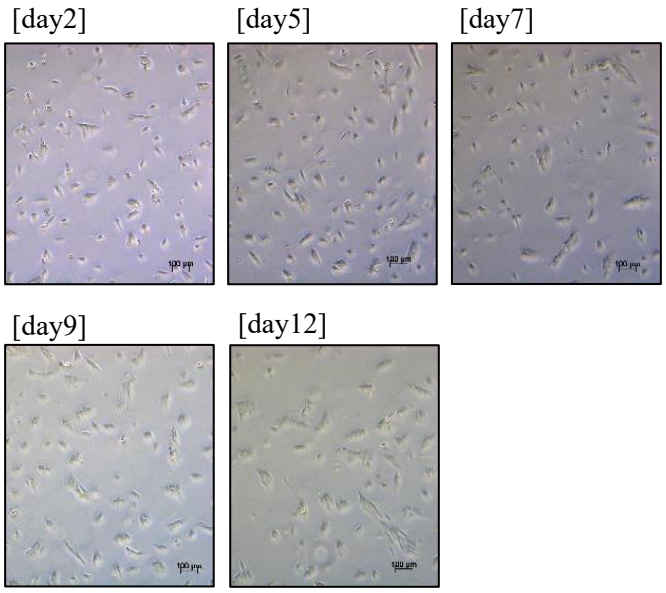
Cancer is a condition in which cancer cells grow in an uncontrolled manner. Most postoperative recurrences of most cancers occur within 5 years after complete resection, however, there are some patients with late recurrence which develop more than 5 years after resection; and this occurs in lung cancer too.

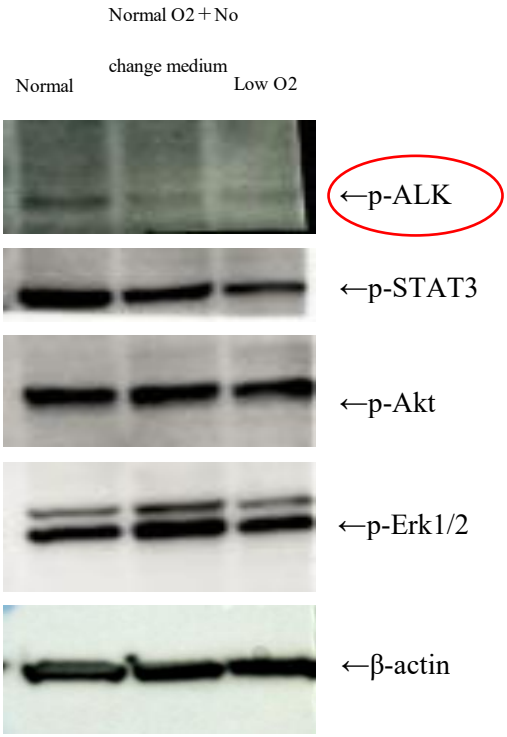
Although there have been several case reports on late recurrence after complete resection for lung cancer, the amount of research that has been carried out to date remains limited and late recurrence of lung cancer is still poorly understood. This may be partly due to the fact that lung cancer has a poorer prognosis than other cancers in addition to many cases recuring at an earlier period. Also, as there are very few basic research reports on tumor dormancy in lung cancer, the detailed mechanisms currently remain unknown.

Recently, it had been reported that most lung cancers that recurred more than 15 years after resection were ALK-positive lung cancers (Cureus. 2023). In patients where recurrence occurred more than 15 years after complete resection it was observed that the tumor likely did not grow at a constant rate over the 15 year period. Furthermore, it is not rare for patients with late recurrence to suddenly detect the recurrence despite years of annual follow-ups. Based on the clinical observations, we hypothesized that late recurrence may be caused by tumor dormancy, in which cancer cells do not proliferate for a long period of time and then grow rapidly. As most lung cancers that recurred more than 15 years after resection were ALK-positive lung cancers, we hypothesize that the ALK gene may be involved in tumor dormancy.

There are three characteristics of cell dormancy: (i) minimum tumor cell proliferation; (ii) minimum tumor cell death; and (iii) reversibility (Cancer Sci. 2019).

As such, we started to culture several lung cancer cell lines under various conditions, and we observed that hypoxic culture conditions suppressed proliferation the ALK-positive lung adenocarcinoma cell lines . No obvious increase in dead cells were observed during culture. Returning the growth environment to an appropriate culture environment also resulted in recovery of cell growth. Therefore, it is clear that the cell culture environment can be manipulated to create a dormant state of ALK-positive lung adenocarcinoma cell lines.





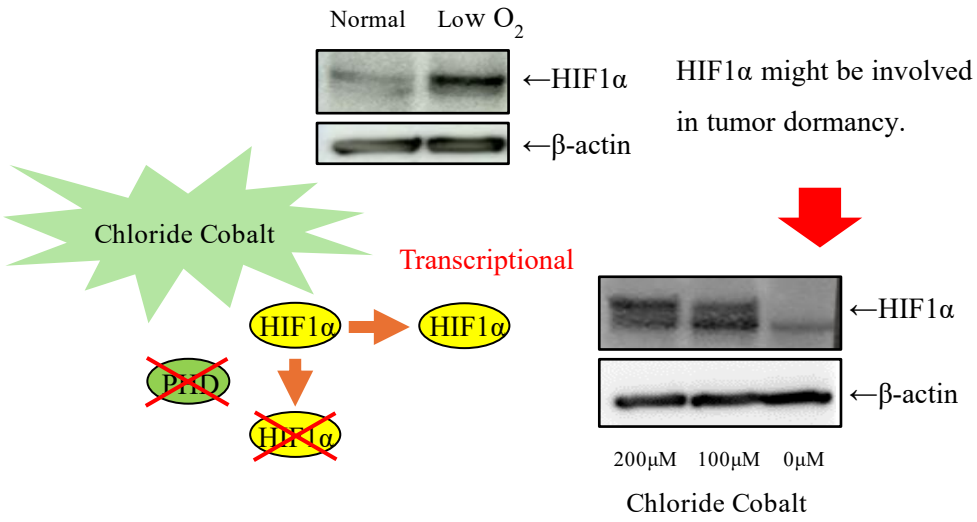
After 12 days of incubation under hypoxic conditions (1% oxygen concentration), the condition was considered dormant. After that, cell growth was initiated once the media had been changed and normoxia was induced.

Figure 1. Reproducing tumor dormancy using cell lines

Furthermore, Western blotting was performed to compare ALK-positive lung adenocarcinoma cell lines in a tumor dormancy state with ALK-positive lung adenocarcinoma cell line cultured under normal conditions, and several signaling pathways were found to be altered, including decreased expression of phosphorylated ALK and increased expression of HIF1α. Among these, we focused on the HIF1α gene as a factor involved in dormancy.

To observe the effect of HIF1α, cobalt chloride was added to ALK-positive lung adenocarcinoma cell line to increase HIF1α expression, which resulted in suppression of cell proliferation and rendered dormant. This suggested that HIF1α may be an important factor in tumor dormancy. Thus, important signals involved in dormancy are slowly being identified.

As society is aging, more patients will likely undergo long-term follow-up after resection. Under such circumstances, it is necessary to appropriately identify patients who need long-term follow-up, and the results of this study could positively contribute to the selection of such patients. Thus reducing the burden for patients in terms of follow-up and also on hospitalization and medical resources.



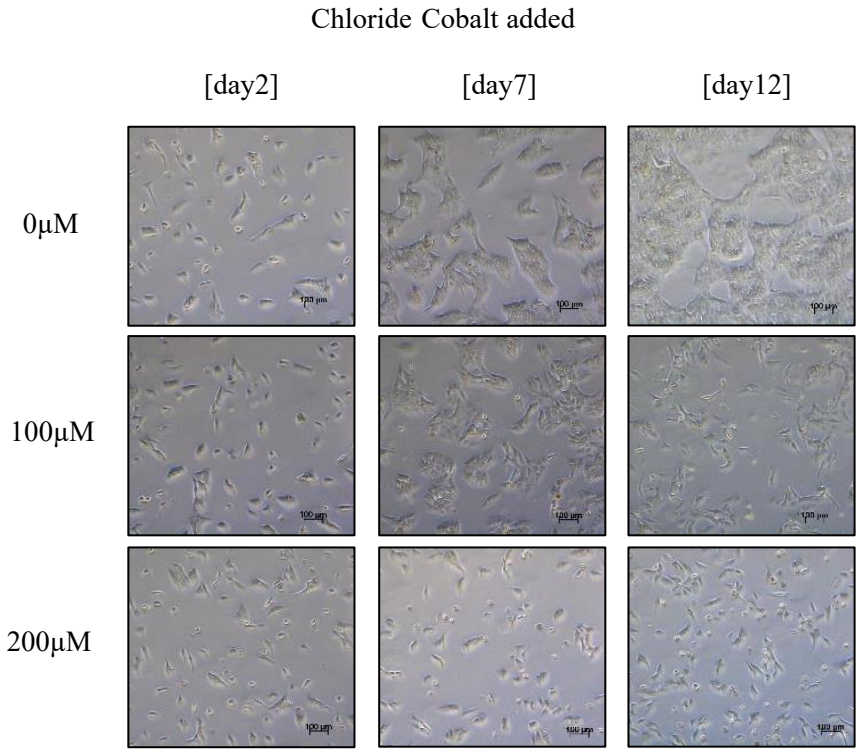


Figure 2. Examining signaling and metabolic changes that occur in a state of tumor dormancy

Additionally, if tumor dormancy can be maintained by altering signals in the tumor, the results of this study may help the development of new therapies to prevent recurrence by confining tumors to a dormant state. Lung cancer is also a cancer for which drug therapy is advancing rapidly, with the advent of molecularly targeted therapies and immune checkpoint inhibitors.

However, drug resistance is a barrier for these therapies, but this is related to the stem cell of the cancer, and studies of tumor dormancy can contribute to the treatment of drug-resistant well as late recurrence.

In summary, this study may lead to innovative advances in the follow-up and treatment selection of lung cancer.

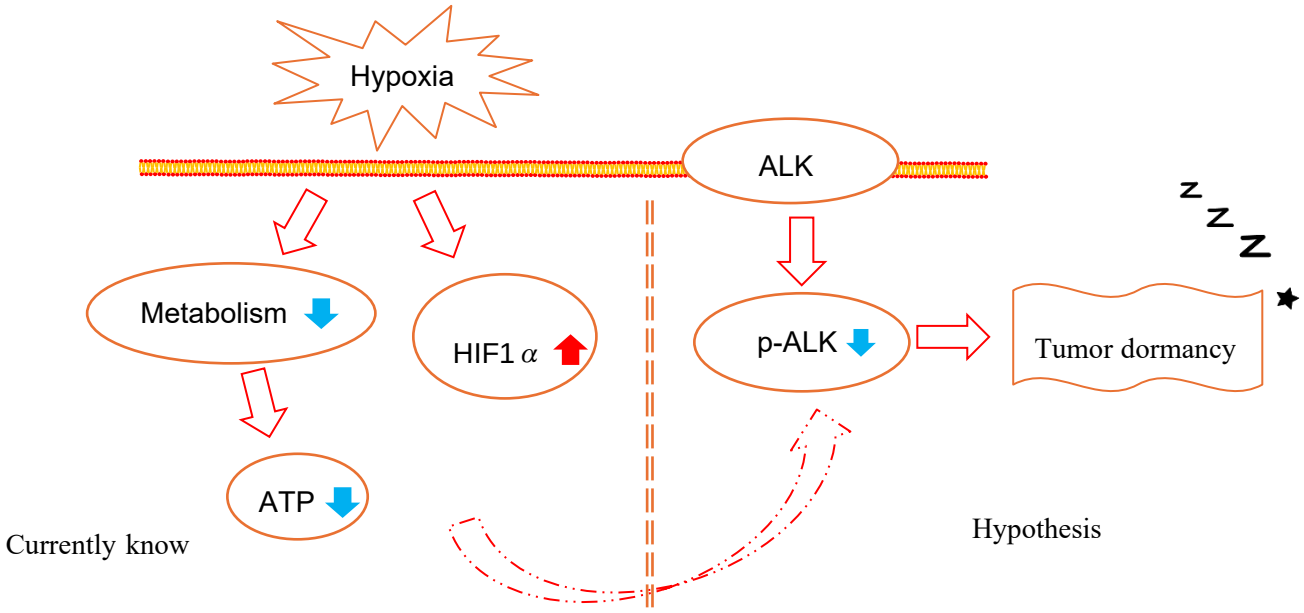


Figure 3. Hypothesis of the mechanism of tumor dormancy

Impact statement

Understanding and manipulating the mechanism of dormancy in lung cancer has three major impacts: 1) it improves patient care by allowing them to obtain the appropriate care relevant to their cancer type, 2) it reduces the healthcare system burden by appropriately categorizing patients and the subsequent follow-up timelines (up to 5 years usually for nondormant, and for possibly dormant cancers a long term follow-up may be recommended), and third, perhaps most importantly this research may lead to the development of improved treatment methods.

The first main impact achieved by examining the characteristics and mechanisms of tumor dormancy, is improved patient care. Being able to identify whether tumors are possibly dormant or not would allow for a more appropriate follow-up schedule and care for their cancer type.

This leads us to the second major impact; the healthcare system. The healthcare system total burden would also be impacted as the detection of the subtype would enable practitioners to categorize their patients for their follow-up needs accordingly too, thus enabling the hospitals to allocate their resources accordingly by selecting patients who require long-term follow-up and those who do not.

The third main impact achieved would be though treatment improvement. This study may lead to the discover of methods for maintaining tumor dormancy by altering signals in the tumor, which may contribute to the development of new therapeutic agents for lung cancer and prevent recurrence by confining the tumor in a dormant state.

Until now, most cancer treatments have aimed to attack and remove the cancer. At present, however, it is difficult to completely remove the tumor after recurrence. This research may lead to a new treatment method in which the tumor is present but prevents it from becoming an obstacle to health.

A public non-scientific summary

Cancer is a disease in which cells grow in an uncontrolled manner. Recently, it is estimated that about 1 in 14 Canadian people will develop lung cancer during their lifetime and 1 in about 20 will die from lung cancer. Even after surgery is performed on a cancer and the entire tumor has been removed, the cancer can recur. This recurrence is not limited to lung cancer, and highlights some of today's treatment limitations.

However, if the patient is treated effectively, a good prognosis is possible even after a recurrence thanks to various effective treatments and drugs. Therefore, it is of the utmost imprtance to detect recurrence early, enabling appropriate treatment.

Most cancer recurrences occur within 5 years after surgery, but some patients may recur more than 5 years after surgery. Cases of lung cancer recurring more than 15 years after surgery have also been reported. One of the problems with cancer recurrence after a long period of time is that many patients have already completed their hospital visits, so the recurrence can go unnoticed and the disease may progress. In this study, we aim to elucidate the mechanism of cancer recurrence after a long period of time.

We hypothesized that the mechanism of recurrence after a long period of time post surgery is that cancer cell proliferation has been 'paused' by some mechanism.

To solve this mystery, we are conducting research using cancer cell lines extracted from a human patient and grown to be used in cancer research. There are many cell lines available for lung cancer. Studies published to date have shown that, in lung cancer, adenocarcinomas in particular can recur, even long after surgery.

As such, various adenocarcinoma cell lines have been cultured under different conditions and onlyone cell line developed a tumor dormancy behavior. Tumor dormancy is defined as a condition in which three conditions are met: (i) minimum tumor cell proliferation; (ii) minimum tumor cell death; and (iii) reversibility. In other words, the tumor does not die but does not proliferate.

In the future, we will use the above selected cell line to further investigate, understand, and modulate the mechanism of dormancy. This will impact lung cancer patients in two large ways.

The first, it will enable us to detect patients who would benefit from a prolonged follow-up.

This would also reduce the burden on hospitals and patients who would not need additional visits beyond the current 5 years post resection period.

The second, it may lead to the development of a new treatment method of dormant cancers by understanding and taking advantage of the tumor dormancy mechanisms to keep them dormant.

In brief, we believe that this research will contribute to lung cancer patients from two perspectives: postoperative patient care and the development of new treatment methods.