Cancer

Persister cells that survive chemotherapy pinpointed

Sumaiyah K. Rehman & Catherine A. O'Brien

A close look at the cells that drive cancer growth after chemotherapy, and thereby contribute to fatal tumour progression, provides new insights into the identity of the cells that manage to survive treatment. **See p.784**

Chemotherapy does not always succeed in tackling a tumour. Understanding how cancer growth resumes after chemotherapy will be key to finding new treatments that might lead to sustainable cures. On page 784, Ohta *et al.*[1] fill in some missing details about the cells that drive such tumour relapses.

The term persister cells originated in bacterial studies to describe bacteria in a 'resting' state that can transiently tolerate antibiotic treatment, although they remain sensitive to antibiotic targeting. This concept of lingering cells that evade treatment has gained prominence in cancer studies, in which these cells are also called persister cells, although they are unrelated to their bacterial namesakes.

Cancer persister cells are non-dividing (quiescent) or rarely dividing (slow cycling) cells that are in a state of reversible resistance to anticancer treatment. They can survive treatment in this persistent state, but if they resume proliferation when use of chemotherapy or a targeted agent ceases, then they remain sensitive to therapy. Crucially, the ability of persisters to escape therapy-induced cell death cannot be explained by genetic mutations. Such drug-tolerant persister (DTP) cells are found in response to a wide range of chemotherapies and targeted anticancer agents. First identified[2] in 2010, persister cells have garnered substantial interest because they represent a potential therapeutic opportunity to target cancer cells before irreversible genetic resistance to treatment occurs. However, many questions about their biology remain unanswered.

Ohta and colleagues sought to determine whether persister cells are pre-existing or form *de novo* in response to chemotherapy. The authors studied human colon cancer cells, growing as miniature organ-like structures called organoids, which were transplanted into mice. The organoid cells expressed the protein LGR5, an established marker of colon cancer stem cells, and these $LGR5^+$ cells were also randomly labelled with fluorescent proteins that enabled individual cellular lineages to be tracked. To study the behaviour of the cancer cells during tumour growth, Ohta *et al.* used a device that enabled them to see cells inside the animal and therefore to track *in vivo* dynamics.

The authors identified a quiescent subset of cells that express the p27 protein present in tumours not yet treated with chemotherapy (Fig. 1). After the onset of chemotherapy, Ohta and colleagues observed an increase in the pool of these $LGR5^+p27^+$ cells. However, it was unclear whether the cells had persisted through chemotherapy or had arisen from cells lacking p27 that responded to chemotherapy by undergoing irreversible cell-cycle arrest and expressing p27.

To address this, Ohta *et al.* tracked cellular lineages using a method that enabled real-time monitoring of cell-cycle regulation. When combined with the live imaging and tracking of individual cells, the data indicated frequent entry into the cell cycle and proliferation of $LGR5^+p27^+$ cells after chemotherapy, whereas most $LGR5^+$ cells lacking p27 did not survive chemotherapy. The authors therefore conclude that $LGR5^+p27^+$ cells are pre-existing dormant cancer stem cells that are responsible for tumour regrowth after chemotherapy ceases.

These results are consistent with the cellular properties reported for previously identified persister cells for a brain tumour called glioblastoma that exhibit stem-cell-like properties and are slow cycling[3]. However, other research groups did not identify pre-existing persister populations for colon, breast and prostate cancer using a different single-cell-tracking technique[4–6]. Moreover, some groups either identified a persister population distinct from cancer stem cells[5] or found a loss of the $LGR5^+$ cancer-stem-cell population in the persister state[7]. Thus, the type of tumour, growth conditions or chemotherapy might influence whether DTPs are found in an untreated tumour. In addition, perhaps what are currently defined as cancer persister cells actually represent cells in various states, which

From the archive

On the trail of viruses that can kill bacteria, and early efforts to assess air pollution.

100 years ago

The ninetieth annual meeting of the British Medical Association was held in Glasgow ... Dr. F. d'Herelle, of the Pasteur Institute, opened a discussion on his theory of "Bacteriophage" — a theory formulated to explain the fact that among the contents of the alimentary canal there always exists a "something" which possesses the power of dissolving bacteria of certain definite types ... This "something" ... is of uncertain origin. ... Dr. d'Herelle ... believes it to be ... an ultra-microscopic enemy of the bacteria.

From *Nature* 26 August 1922

150 years ago

Air and Rain. By R. A. Smith — This work contains the germs of a system of chemical climatology. It indicates a plan of testing the purity of the atmosphere of localities with regard to certain constituents of organic origin — the *débris* of living things — by washing the air, and determining the character and amount of the substances in solution by certain micro-chemical methods. By the systematic repetition of these testings, the possibility is foreshadowed that we may be enabled to classify such atmospheres, and actually to assign to them quantitative sanitary values. It thus points out how we may be able to estimate the difference between the vitiated air of the town and the pure air of the country. Our senses and experience tell us plainly of the existence of such differences; but chemistry has been hitherto powerless to detect them. ... Cavendish, nearly a century ago, asserted that chemical experiments could not distinguish the air of London from the air of the country ... this assertion seems as true of to-day as it was of the time when uttered ... [C]hemists, in judging of the quality of the air of any locality, have been obliged to content themselves with determining the proportion of oxygen and carbonic acid which it contains, in conformity with the practice of their ancestors of a century back.

From *Nature* 22 August 1872

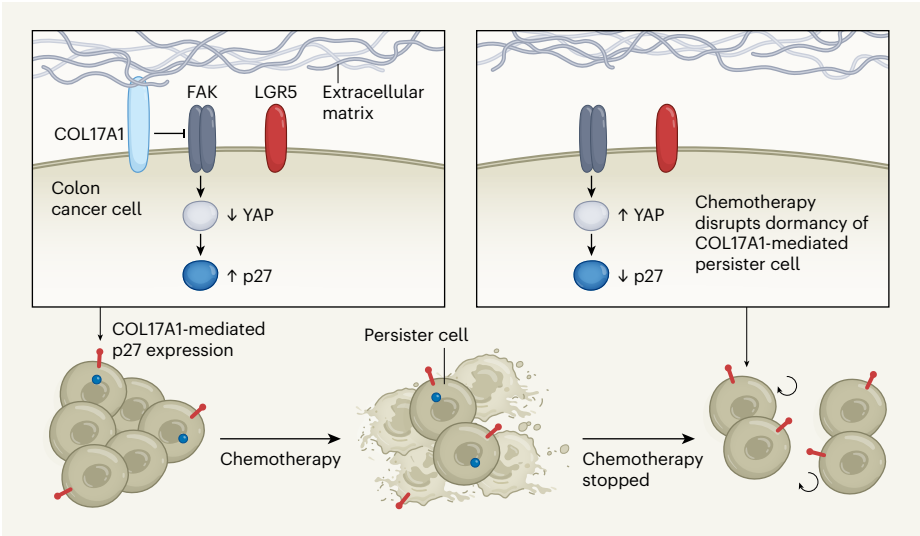

Figure 1 | Cancer cells that survive chemotherapy. Ohta *et al.*[1] studied human colon cancer cells transplanted into mice to investigate cells, termed persister cells, that survive chemotherapy in a 'dormant', non-dividing state. The authors focused on cancer stem cells that express the protein LGR5. A subset of these cells that give rise to persister cells were present even before chemotherapy commenced. A hallmark of these persister cells is expression of the protein p27. The authors report that p27 expression depends on a pathway mediated by the receptor protein COL17A1, which binds to the extracellular matrix surrounding the cell. COL17A1 inhibits the receptor FAK and thereby suppresses the pathway mediated by the protein YAP, and these inhibitory actions lead to p27 expression and dormancy. Chemotherapy kills tumour cells, apart from persister cells, but COL17A1 degradation occurs as a consequence of chemotherapy. This leads to activation of the FAK and YAP pathway and disrupts dormancy. The surviving cells stop expressing p27 and begin to proliferate, resulting in tumour relapse.

would explain some differences regarding pre-existing compared with *de novo* persisters and their relationship to cancer stem cells. It also remains to be determined whether persisters are in part defined by other external factors, including the type of therapy administered and the tumour micro-environment.

Relating to the role of the tumour micro-environment, Ohta *et al.* made a key discovery with their observation that most of the LGR5$^+$p27$^+$ cells lost p27 expression when the cells were detached from the culture plate *in vitro*, which enabled the cells to undergo cell division. The authors found that interactions between the cell and the extracellular matrix — a network of proteins and molecules that surround and support the cells in the body — was key to maintaining the persister state. Gene-expression profiling pointed to the protein COL17A1 as having a crucial role in maintaining the dormant state of LGR5$^+$p27$^+$ cells. COL17A1 is a component of hemidesmosomes, structures that enable cells to adhere to the extracellular matrix.

The authors report that COL17A1 regulated p27 expression independently of LGR5 status. This represents a new finding for the field because it indicates that persisters are defined in part by their interaction with their micro-environment. Future work will be needed to gain a deeper understanding of how the micro-environment, including intratumoral immune cells, influences the DTP state.

Ohta *et al.* demonstrate that cancer cells are held in a dormant state through COL17A1-dependent repression of a pathway containing the proteins FAK and YAP, and that this dormancy system is disrupted by chemotherapy, which enables tumour cells to grow again. Furthermore, when the authors used a small-molecule inhibitor to target YAP in organoids engineered to lack COL17A1, this resulted in p27 re-expression, suggesting that YAP-pathway inactivation has a key role in maintaining colorectal cancer cells in a persister state. Interestingly, chemotherapy resulted in remodelling of the extracellular matrix, loss of COL17A1 expression, activation of the FAK–YAP signalling pathway and, consequently, an increase in the number of cells re-entering the cell cycle (and a decrease in LGR5$^+$p27$^+$ cells). Indeed, YAP inhibition delayed, but did not prevent, tumour regrowth, suggesting that YAP has a role in tumour regrowth after chemotherapy by preventing LGR5$^+$p27$^+$ cells from exiting dormancy. However, this delay (but not prevention) indicates that much remains to be understood about the survival mechanisms used by persisters.

"Persister cells are defined in part by their interaction with their micro-environment."

Numerous cancer models demonstrate a role for YAP in persister cells, and it seems to have different functions depending on the persister model investigated. One study[7] of a colorectal cancer model indicates that chemotherapy imposes quiescent persister characteristics on cancer cells that depend on the upregulation of the protein YAP1. And an *in vivo* study[8] reported data suggesting that, in the context of lung cancer, persisters induced by targeted therapies depend on the activation of YAP1. Finally, a study[9] that harnessed expression of an RNA-binding protein called Mex3a as a marker of persister cells in colorectal cancer identified upregulation of the YAP pathway as a key feature of persister cells. Thus, the findings of three independent groups all indicate that persister cells are defined by upregulation of the activity of the YAP pathway. Intriguingly, Ohta *et al.* found that YAP downregulation was required for the maintenance of dormancy, thereby illustrating the complexity of persister biology.

The findings related to YAP raise another important topic, that of how to target persisters. One option, as described by Ohta *et al.*, is to maintain persisters in the dormant state. However, research[10] has demonstrated that, over time, persister cells acquire resistance to treatment through diverse mechanisms, suggesting that the dormant state provides a reservoir of cells for the emergence of drug-resistant cells driven by the acquisition of irreversible genetic mutations. Thus, long-term *in vivo* studies are needed to determine whether strategies designed to lock cancer cells in the persister state represent a solution that can be sustained over time. Other approaches under investigation are to eliminate persisters or to try to prevent their formation.

Sumaiyah K. Rehman is at the Princess Margaret Cancer Center, University Health Network, Toronto, Ontario M5G 1L7, Canada. **Catherine A. O'Brien** is in the Department of Surgery, University Health Network, Toronto, Ontario M5G 1L7, Canada, at the Princess Margaret Cancer Center, and in the Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto. e-mail: cobrien@uhnresearch.ca

1. Ohta, Y. *et al. Nature* **608**, 784–794 (2022).
2. Sharma, S. V. *et al. Cell* **141**, 69–80 (2010).
3. Liau, B. B. *et al. Cell Stem Cell* **20**, 233–246 (2017).
4. Dhimolea, E. *et al. Cancer Cell* **39**, 240–256 (2021).
5. Echeverria, G. V. *et al. Sci. Transl. Med.* **11**, eaav0936 (2019).
6. Rehman, S. K. *et al. Cell* **184**, 226–242 (2021).
7. Solé, L. *et al. Nature Commun.* **13**, 2866 (2022).
8. Kurppa, K. J. *et al. Cancer Cell* **37**, 104–122 (2020).
9. Álvarez-Varela, A. *et al. Nature Cancer* https://doi.org/10.1038/s43018-022-00402-0 (2022).
10. Ramirez, M. *et al. Nature Commun.* **7**, 10690 (2016).

The authors declare no competing interests.
This article was published online on 12 July 2022.