A shining light in the fight against childhood leukaemia?

Leukocytes (or white blood cells) represent the body’s first line of defence against infection, and an effective immune response depends largely on the timely mobilisation of sufficient leukocytes to inflamed tissues. Leukocyte targeting to infection sites is orchestrated by a group of cell adhesion receptor proteins that are present on leukocyte surfaces. The α4-integrins represent one group of cell adhesion receptors that are highly expressed by leukocytes, and the role of integrins in leukocyte adhesion, migration and resistance to chemotherapy has been the focus of Dr Lim’s research. Leukocyte targeting is fine-tuned via an intricate balance in the interactions between these cells and the environment from which they originate, namely the bone marrow stroma. The stroma generates signalling cues and growth factors for blood cell formation (a process called haematopoiesis) in all types of blood cells, e.g., red blood cells and platelets, in the bone marrow.

Dr. C. James Lim, PhD, is an Associate Professor at the Department of Pediatrics, University of British Columbia, as well as Investigator at the Michael Cuccione Childhood Cancer Research Program at British Columbia Children’s Hospital, Vancouver, Canada. His research group aims to unravel the mechanisms underlying acquired resistance to chemotherapy in childhood malignancies, with the goal of identifying new treatment strategies to improve the odds for children fighting this disease.

ALL OUT OF CONTROL

Failures in the processes that regulate leukocyte targeting can lead to leukaemia and other immune diseases. Leukaemia refers to a group of blood cancers that often begin in the bone marrow, and are characterised by very high numbers of lymphoblasts, essentially abnormal and underdeveloped white blood cells. Acute lymphoblastic leukaemia (ALL), Dr Lim points out that although treatment outcomes are very good for childhood leukaemia – with a success rate of about 85% – there is concern for the remaining 15% of cases that relapse with drug-resistant forms. Treatment-resistant relapsed leukaemia remains the leading cause for childhood disease-related mortality.

INTEGRINS IN CHEMoresISTANCE

A small number of cancerous cells that don’t succumb to initial chemotherapeutic intervention may persist as minimal residual disease. These intransigent cells represent the most likely starting point for relapsed cancers, which tend to be multidrug-resistant in a manner not limited to the agents used in initial chemotherapy. Dr Lim’s group examines differences in cellular signalling between healthy and tumour cells in order to find clues about how chemoresistance gains a foothold, and how exactly the α4-integrins are involved in that process. Studies from other research groups showing that interactions between integrins and associated proteins were implicated in enhancing tumour cell pro-survival signalling and chemoresistance, in a process known as cell adhesion-mediated drug resistance (CAM-DR).

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Dr Lim’s group first compared the response to chemotherapy in ALL lymphoblasts that either express α4-integrins, or are engineered not to. When exposed to the widely used chemotherapeutic, doxorubicin, leukemic cells expressing α4-integrins and adherent to the integrin substrate were more resistant to elimination by doxorubicin compared to those without α4-integrins, thereby supporting a direct role for α4-integrins in CAM-DR in ALL. In a series of elegant experiments, the group then showed that expression of a conserved region (known as a peptide motif) common to all α-integrins was sufficient to promote CAM-DR in ALL cells, and this process involves the coordinate influx of calcium into and the efflux of doxorubicin out of the cells. In essence, a process initiated by cell adhesion via α-integrins enhances the removal of doxorubicin from the cells via molecular pumps, effectively reducing the drug’s dosage within the tumour cell and its efficacy.

The discovery by Dr Lim’s group that CAM-DR is not limited to a particular integrin or type of leukemia provides hope that future attempts to target CAM-DR with new therapeutic agents will not only improve treatment outcomes for leukemia, but a broad spectrum of blood cancers.

ENLISTING THE IMMUNE SYSTEM

The chemotherapeutic toolbox is rather...
A key feature of ICD is the presentation of a protein called calreticulin on the surface of cells treated with drugs that stimulate ICD. This cell surface calreticulin then serves as an ‘eat me’ signal for macrophages, a type of specialised white blood cell, to engulf and destroy cancer cells in a process known as phagocytosis.

**SOLVING THE ‘EAT ME’ MYSTERY**

Researchers investigating ICD were fascinated by how calreticulin, which is normally restricted to one particular compartment inside the cell (the ER or endoplasmic reticulum), reaches the cell surface during ICD. Dr Lim’s research group shed some light on this mystery when they discovered that calreticulin, released from the ER during periods of drug-induced ER stress, moves through the otosolic compartment prior to its appearance on the cell surface. While in the otosolic, calreticulin can interact with the otosolic part of α-integrins, and this interaction only occurs when integrins are activated; either by cell adhesion or upon stimulation with integrin agonists. This means that when integrins are activated, they bind calreticulin, thus trapping it inside the cell, and the ‘eat me’ signal (cell surface calreticulin) is reduced. This finding, reported in a 2016 publication, is the first to demonstrate a direct role for integrins and cell adhesion in diminishing the likelihood of ICD, and have implications in our understanding of effective immunotherapy.

**TARGETING THE TUMOUR MICROENVIRONMENT**

Once leukaemia has developed, its continuous growth and ability to metastasise (or spread) depends on interactions between the leukaemic cells and their surrounding cellular environment, often called the tumour microenvironment (TME). Within the TME, the bone marrow stroma represents a niche for leukaemic cells to interact with microenvironmental factors (e.g., cell adhesion factors such as integrins, which promote their survival and contribute to drug resistance).

The findings from Dr Lim’s group to date support the notion of the TME being a safe haven for cancer cells, and highlight the TME as a promising target for the therapeutic intervention of leukaemia.

**UNANSWERED QUESTIONS**

Based on Dr Lim’s findings to date, it is conceivable that blocking integrin activity of immune cells to seek and detect foreign invaders such as bacterial pathogens. Given the obvious importance of immune-surveillance, novel strategies that can selectively target α4-integrin signalling in the context of CAM-DR are preferable over complete α4-integrin inhibition.

The exact source of the protective signals that allow tumour cells to acquire chemoresistance is not yet known, but it is likely that the bone marrow stroma

Some chemotherapeutic drugs, including doxorubicin, don’t just kill tumour cells, they also stimulate the immune system to eliminate cancer cells in a sophisticated process known as immunogenic cell death