Unlocking the mechanism of action of arsenic trioxide in leukaemia

The blood cancer acute promyelocytic leukaemia (APL) has recently been transformed from a fatal condition to a curable one, thanks to the development of treatments including vitamin A1 and arsenic trioxide (ATO). Although very effective, the mechanism of action of ATO has remained unclear until now. Changing this is Professor Paul B Tchounwou and his team at Jackson State University, USA, focus on understanding the mechanisms of action of ATO. These effects were evaluated by reducing the expression of E2F1, a cell-cycle regulating factor that, when in abundance, can lead to the formation of cancer (tumorigenesis). It’s also known that changes in the levels of E2F1 can lead to apoptosis, through a specific biochemical pathway that includes the mediation and function of the very important tumour suppressor protein p53 (p53-dependent pathway). P53 suppresses the formation of cancer cells in our bodies by protecting and promoting the repair of our DNA.

The cell-cycle consists of a series of events that lead to the division of a cell into two new cells. It’s a complicated process with many modulating factors, including a family of proteins called the cyclins. Cyclic E controls the progression of a cell through its cell cycle. More specifically, the presence of cyclic E causes a specific chemical reaction (phosphorylation) to a protein called retinoblastoma protein (pRb), which in turn gets deactivated – an action that leads to the division of the cell and therefore its proliferation. Over-expression of cyclic E has been shown to correlate with tumorigenesis. pRb is a tumour-suppressing protein that is dysfunctional in some cancers, especially since one of its functions is to prevent excessive cell growth by blocking the progress of the cell cycle until the cell is ready to divide. These two cell-cycle regulating pathways are also connected via the E2F1 factor, providing a functional relationship between the two regulating proteins p53 and pRb.

Many more important molecules control the cell cycle, including phosphoinositide 3-kinase (PI3Ks). PI3Ks are a family of enzymes that catalyse chemical reactions (phosphorylation) necessary for cell functions, such as cell growth, cell proliferation, and survival, and therefore also play a major role in tumorigenesis. More specifically, activation of PI3Ks leads to increased E2F1 expression.

UNLOCKING THE ACTION OF ATO

The Jackson State University team conducted experiments using a human immortalised APL cell lines, a population of human cancer cells that divide indefinitely, to study the effects of ATO. These effects were evaluated by measuring some of the factors and components that participate in the process of cell proliferation and cancer growth (when out of balance) to investigate the exact pathways ATO acts upon.

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The researchers are shedding light on the complex pathways through which ATO blocks the multiplication of cancer cells. Their work offers insights into the development of novel therapies for the management of human cancers.

Leukaemia is the name of a group of blood cancers, which often begin in the bone marrow and result in high numbers of abnormal blood cells. A combination of genetic and environmental factors, including exposure to specific chemicals and radiation, are known to be involved in the development of these conditions. There are four main types of leukaemia: acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), chronic lymphocytic leukaemia (CLL), and chronic myeloid leukaemia (CML). In adults, CLL and AML are most common, with AML demonstrating the lowest survival rates. The treatment of leukaemia may often involve a combination of chemotherapy, radiation therapy, targeted therapy, and bone marrow transplant.

Acute promyelocytic leukaemia (APL) is a subtype of AML. APL forms inside bone marrow cells as a result of changes between chromosomes 15 and 17, called translocations – a type of chromosomal abnormality in which a chromosome breaks apart and then partially reattaches to a different chromosome. This chromosome rearrangement leads to the creation of two fusion genes (two genes that are joined so that they act as a single unit): the promyelocytic leukaemia gene (PML–RARα) and the retinoic acid receptor alpha gene (PML–RARα). PML–RARα is often used as a marker for the diagnosis of the disease. PML–RARα is the gene responsible for the symptoms of APL and is the target of drug therapy using arsenic trioxide (ATO). Along with all-trans retinoic acid, a metabolite of vitamin A1, ATO is the standard treatment for APL, a breakthrough that has meant rates of remission and survival rates are now high. However, its mechanism of action has not been – up to now – fully understood. Also, a few cases of APL that show resistance to ATO have recently been identified. Motivated to uncover the obscure way ATO works in our bodies, Professor Paul B Tchounwou and his team at Jackson State University, USA, focus on understanding the mechanisms of action of ATO in human cells and unlocking potential new treatments.

ATO AFFECTS CELL CYCLE REGULATION

ATO is an inorganic compound that has the chemical formula As2O3. ATO is also used as a medication to treat APL by altering the function of cells, eventually blocking the multiplication of blood cancer cells. Proposed mechanisms of action include the induction of apoptosis (the natural process of cell death), and the inhibition of angiogenesis (the formation of new blood vessels that supply cancerous growths).
Cyclin E is required for initiation of DNA duplication. The results showed that treating APL -specific antibodies that target E2F1. and phosphorylation of PI3K signalling molecules in the cell lines – blocking cancer growth. '-' Tchounwou explains, ‘ATO also modulates the interaction and association of E2F1, pRb, and p53 proteins by phosphorylation of Rb at specific points. In doing so, it reduces the phosphorylation of phosphoinositide 3-kinase (PI3K) signalling molecules, and abolishes the viability of cancer cells.’ The team’s results show that ATO treatment works by affecting both p53 and PI3K signalling pathways, particularly as it induces the accumulation and activation of the DNA protecting protein p53 and simultaneously reduces the phosphorylation of most of PI3K signalling molecules in the cell lines – blocking cancer growth. For the first time, Tchounwou and his team show that ATO inhibits the multiplication of cancer cells through the reduced expression of E2F1 and cyclin E, and the stimulation of pRb phosphorylation. This suggests that ATO’s effects are mediated by the tumour protein p53.

This newly found mechanism of action could prove very important – providing a new molecular target for research into novel drug treatments.

Three types of APL cells were used: NB4, KG-1a, and HL-60 cells. Initially, NB4 and KG1a cells were treated with ATO for 24 hours at 37°C inside a CO2 incubator. After incubation, the cells were collected, and analysed by Western blotting – a method to detect the levels of E2F1, cyclin E, and pRB proteins. Next, APL cells were grown with and without ATO, before carrying out immunohistochemistry to identify levels of the E2F1 protein using specific antibodies that target E2F1. Finally, NB4 cells were treated with different concentrations of ATO for 24 hours, which were then analysed through the technique of chromatin immunoprecipitation (CHIP). CHIP is a method used to assign specific genes to their respective proteins – and in this case to calculate the expression of E2F1 and pRB factors following treatment with different concentrations of ATO.

PS3 AND PI3K SIGNALLING PATHWAYS
The results showed that treating APL cells with ATO reduces the expression of both cell-cycle factors E2F1 and cyclin E. This was observed in all three cell types by stimulating the expression of the tumour suppressor pRB protein in a concentration-dependent manner. The immunohistochemistry experiments revealed that while ATO stimulates a localised increase in levels of pRB, it also reduced the expression of E2F1 transcription factor and cyclin E protein, and the phosphorylation of PI3K.

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