

Uncontrolled intracellular osmotic pressure leads to cancer

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Abstract

At present more than 9 million people die of cancer every year. Simple and broad-spectrum drugs are still an urgent need for cancer patients. Recently, we proposed a new hypothesis that intracellular osmotic pressure (IOP) is the driving force of cell division, and abnormal tumor proliferation is the result of uncontrolled IOP in cells. On the one hand, aneuploidy and abnormal function of Na^+/K^+ pump lead to a faster rise of IOP in tumor cell than normal cells, on the other hand, abnormality of cytoskeleton assembly leads to the decrease of tolerance limit of cell membrane (TLCM) of tumor cells for resisting IOP. This hypothesis predicts: 1) Tumor cells were more intolerant to hypotonic stress than normal cells. 2) Malignancies may be selectively killed by a sudden increase of IOP and combined with decrease of the TLCM of tumors. Na^+/K^+ pump inhibitors can promote to increase the IOP of tumor cells and cytoskeleton inhibitors can dramatically lower the TLCM of tumor cells. Therefore, Na^+/K^+ pump and cytoskeleton inhibitors may have a synergistic effect to kill tumor cells. 3) Molecules regulating cell osmolality may be new targets for cancer treatment.

The latest statistics show that 18 million new cancer cases and 9.6 million deaths occur each year in the world (1). At present, targeted drugs and immunotherapy have made great breakthroughs(2). However, due to the heterogeneity of gene mutations of tumor cells(3, 4), molecular diagnosis and targeted therapy have become more and more complicated. Moreover, there are no specific targeted drugs for quite a number of malignant tumors (4-6). So, simple and broad-spectrum cancer treatment is still an urgent need.

Tumor cells are characterized by abnormal proliferation. Deep understanding of the mechanism of cell proliferation is the basis of exploring the mechanism of carcinogenesis. Traditional theory views that growth factor is the promoter of cell proliferation⁽⁷⁾. However, there are still some problems that cannot be explained. For example, the growth of bacteria and plants is not as dependent on growth factors as mammalian cells^(8, 9), so there may be another explanation for the mechanism of cell proliferation.

Recently, we proposed a new hypothesis that uncontrolled intracellular osmotic pressure (IOP) leads to cancer based on the semi permeability of cell membrane⁽¹⁰⁾, This hypothesis holds that IOP is the driving force of cell proliferation, and abnormal tumor proliferation is the result of uncontrolled IOP of cells. We believe that verification of this hypothesis may not only deepen our understanding of the mechanism of cell proliferation and carcinogenesis, but could also explore new methods for cancer treatment.

IOP is the driving force of cell division

Cell structure is complex, which often confuses us. Recently, we try to understand the mechanism of cell division physically. The cell membrane is a lipid bimolecular layer, which could form a spherical structure with a diameter of about 10-30 nm in water. The cell membrane is semi permeable. Water molecules and lipids pass through the cell membrane more easily than macromolecules and ions ^(11, 12). Because the molar concentration of substances inside cells is generally greater than outside cells, the IOP is generally greater than extracellular osmotic pressure (EOP) of cells. Indeed, the IOP in bacteria, fungi and plants often reaches 4-5 atmospheric pressure ^(9, 13, 14). What's more, contrary to the general view that the IOP and EOP of mammalian cells are equal. Some phenomena suggest that the IOP and EOP of mammalian cells is not naturally equal. For example, the cells will swell for a short time of ischemia and hypoxia, and If longer, the cell could even necrosis. Therefore, the maintaining balance of the IOP and EOP of mammalian cells rely on energy consumption. In fact, mammalian cells mainly rely on cytoskeleton actin and Na^+/K^+ pump to resist IOP, and the assembly of actin and the work of Na^+/K^+ pump require energy consumption ⁽¹⁵⁻¹⁷⁾. In vitro experiments show that 20-70% of the ATP in cells is used to maintain the balance of IOP and EOP of mammalian cells ^(15, 16). Therefore, if mammalian cells do not actively regulate the balance of IOP and EOP, they will burst quickly like soap bubbles. Only by controlling the the balance of IOP and EOP, the cells may not be swollen and necrotic. On the other hand, since IOP has

such a great power, it is possible for cells to use the power of IOP to complete the process of cell division, and as a result IOP is no longer the destructive force but the power of cell division(10). This hypothesis is supported by the experiment of AFM: the pressure on the cell membrane increases continuously during the process of cell division, rising from the G0 stage to the highest point at the M stage. With cell division, the pressure drops rapidly (17). Therefore, we believed that increase of IOP is the driving force of mammalian cell division. The fundamental reason for periodically division of cells is to periodically lower the IOP.

Increase of IOP promotes cell swell and cell division

If IOP is considered to be the driving force of cell division, it must meet at least two conditions: 1) promotion of cell division and 2) initiation of DNA replication. Early studies have shown that bacteria, fungi, and plant cells swell before they start to divide, and the power of swelling comes from IOP (13, 14, 18-20). Recently, it has been found that in the process of mammalian cell division, the mammalian cells also swell at first and then divide(17, 21). It is interesting that the volume of cells after division is close to that before division. What causes cells to keep the same volume? Because the value of tolerance limit of cell membrane (TLCM) is constant, when IOP reaches the value of TLCM, cells must divide, if not, they will burst and die. Therefore, cell division may only be an active way to avoid bursting before division. In this way, when IOP rises to the value of TLCM, it is the time point of cell division. In the same

environment, the value of TLM of same type cells is near, so the size of these cells is similar. Similarly, in different environments, different types of cells have different TLM, so there are differences in cell size. Therefore, increase of IOP promotes cell swell and cell division. In fact, some experiments indirectly support this hypothesis. For example, proper low osmolarity stress can promote cell proliferation(22), and the low concentration of Na⁺/K⁺ pump inhibitor can initiate DNA replication of neuron (23), while the high osmolarity environment inhibits cell proliferation (24).

Increase of IOP expand nuclear space for initiation of DNA replication

The existence of binucleate, multinucleated cells and meiosis suggest that DNA replication and cell division are two independent events, both theoretically and experimentally (25, 26). However, it is clear that only when a synergistic relationship between DNA replication and cell division is established can cells survive. For most cells, DNA must complete at least one round of replication before cell division. However, does DNA replication initiate cell division or does cell division initiate DNA replication? If DNA replication initiates cell division, DNA replication should not be less than one cycle before cell division. So there should be cells with multiple cycles of replication. However, up to now, almost all eukaryotic cells, including fungal, plant and animal cells, have only one cycle of replication before each cell division. Although in a nutrient-rich environment, bacterial cells can initiate multiple rounds of replication before division, only one round of replication is completed

before cell division (13). Therefore, DNA replication initiated by cell division may be more reasonable. How does cell division start DNA replication? There may be at least two mechanisms, one is chemical signaling mechanism and the other is physical mechanics mechanism. As IOP promotes cell volume expansion, the membrane will release chemical stress signals under mechanical pressure, and the stress signals will increase with the rise of IOP. These chemical stress signals will initiate DNA replication through signal pathway. In fact, substantial experimental evidence support this suggestion ⁽²⁷⁾.

Under pathological conditions, the cell nucleus will shrink due to the contraction of nuclear membrane and nuclear matrix. For example, apoptotic and necrotic cells show nuclear pyknosis. Therefore, in physiological state, IOP may play an important role in providing the physical space for DNA to function. Nuclear space of eukaryotic cells is limited. Without extra space, DNA may not be able to replicate (28-31). Therefore, IOP promotes the cell volume to expand during the process of cell division, meanwhile, through the cytoskeleton to pull the cell nuclear membrane, promotes the cell nuclear volume to increase, provides space for DNA replication. Therefore, DNA replication needs not only a certain concentration of chemical stress signals, but also the physical space. In addition, it was found that bacterial DNA replication initiation sites are directly linked to cell membrane, while eukaryotic DNA replication initiation sites are linked to nuclear skeleton or nuclear matrix (32, 33). Because DNA replication needs to open DNA double strands, there

may be a possibility: the expanding volume of cell directly or indirectly pulling DNA through the cytoskeleton and nuclear matrix, which may help to open DNA double strands and trigger DNA replication. Although there is no evidence that IOP can directly initiate DNA replication, a large number of experiences shows that physical traction can promote cell division, such as skin traction to expand the area of skin graft (34, 35). Therefore, the increase of IOP not only provides physical space for DNA replication, but also may directly initiate DNA replication by mechanical pull. In conclusion, IOP is the driving force of cell division because it can promote cell division and initiate DNA replication.

IOP and cytoskeleton regulate cell differentiation by changing cell morphology

Limited space can limit DNA replication, as well as RNA transcription. Although it is clear that the production of different types of cells during development is regulated by signal molecules and transcription factors (36), the maintenance of different cell types is controlled by epigenetic modifications (37, 38). However, it is not clear whether physical factors could affect gene transcription. Animal cell types are diverse, each of which has different three-dimensional geometry. The difference of cell morphology indicates that the cell nucleus is pulled by the cytoskeleton in various directions of three-dimensional, which leads to different shapes of nucleus⁽³⁹⁾. Many results showed that the binding site of DNA and nuclear matrix was active in gene transcription⁽⁴⁰⁾, therefore, it can not be ruled out that IOP and cytoskeleton

work together to regulate the shape of nucleus, resulting in different physical space sizes in different directions, thus affecting gene transcription level in different directions of DNA, and ultimately affecting cell differentiation. If so, the constant changes in cell morphology during development may also play an important role in the production and maintenance of cell types. Therefore, IOP and cytoskeleton can not only change cell morphology, but also indirectly affect gene transcription and cell differentiation (41, 42). Recently, it was found that the genes of transcriptional activation in nucleus will move from transcriptional silencing region to transcriptional activation region(43). A typical example is Hox gene clusters. Using 4C-seq, the Duboule laboratory observed that the activated hox genes shift from an inactive chromatin domain to an active domain during the development the embryonic axis(44). Although the mechanism of position change of Hox gene is not clear, we speculate that it may be the result of physical traction with nuclear morphological change.

Delaying or inhibiting cell division costs more energy

In a nutrient-rich environment, the cell cycle of bacteria is usually 40 minutes but that of plant cells takes more than 20 hours. The thickness of bacterial cell wall is about 10 nm, while that of plant cell is at least 100 nm (9, 13, 14). This suggests that delaying cell division requires greater endurance to fight the rise of IOP. In line with this, inhibiting mammalian cell proliferation also requires more energy. Mammalian cells are generally divided into three types. The first is unstable cells, such as skin and intestinal epithelial cells, which are

always in a state of division. The second is stable cells, such as liver cells, which are generally not obviously divided. The third is permanent cells, such as cardiomyocytes and neurons, that is, in any case, they no longer divide. Mammalian cells generally adopt two ways to control IOP. The first is cytoskeleton, which fight against the swelling force caused by IOP in cell. The second is Na^+/K^+ pump, which pump out three sodium ions and in two potassium ions at a time, lowering the ionic osmotic pressure inside the cell, and slowing down the increase of the overall IOP. Substantial evidence have shown that the level of unstable cell membrane potential is low (10-30 mV), while that of the stable cell membrane potential is medium (40-50 mV), and that of the permanent cell membrane potential is the highest (60-70 mV). The membrane potential reflects the energy consumption against IOP by Na^+/K^+ pump ^(45, 46). Therefore, it takes more energy to inhibit or slow down cell division.

Uncontrolled IOP of cells leads to cancer

Cancer is a disease of uncontrolled cell proliferation. When IOP reach to TLCM, the cell begins to divide, and if not, it will burst and die (10, 13). Therefore, the speed of cell division is mainly determined by two factors: the acceleration of IOP and the value of TLCM. If there exists any factors which could increase the acceleration of IOP and/or decrease the value of TLCM, then the cells would divide prematurely. Due to the time of DNA synthesis and repair cannot be shortened at will, premature division of cells before

completion of DNA replication or repair would lead to increased gene mutation rate, genomic instability, uneven chromosome distribution, karyotype aneuploidy, vicious cycle, and finally cancer. In fact, many studies demonstrated that the number of DNA replication initiation sites in tumor cells increase significantly compared with normal cells, suggesting that tumor cells are indeed in a state of premature division(47-51). Therefore, many genetic changes in tumor cells can be attributed to premature division caused by uncontrolled IOP.

There are at least two lines of evidence showing that the acceleration of IOP in tumor cells is greater than that in normal cells: 1) the membrane potential of tumor cells is lower than that of normal cells ^(45, 52). Moreover, many studies have shown that the concentration of Na⁺ in tumor cells is 2-5 times higher than that in normal cells ⁽⁵²⁻⁵⁴⁾. These indicated that the function of Na⁺/K⁺ pump in tumor cells decrease. 2) Most tumor cells are aneuploid karyotype, and the number of chromosomes is generally more than that of normal cells (55, 56). In equal time, aneuploidy produces more RNA and protein and increase the acceleration of IOP in tumor cells compared with normal cells, thus initiating tumor cell division and DNA replication in advance ^(10, 57).

On the other hand, many lines of evidence show that the value of TLCM of tumor cells to resist IOP is decreased. 1) Histological and morphological studies showed that the cytoskeleton of tumor cells was obviously abnormal

(58-68). 2) Transgenic experiment showed that oncogene products such as Src, Ras and Rho could directly disrupt the assembly of cytoskeleton⁽⁶⁹⁻⁷¹⁾. 3) Some studies have shown that tumor suppressor protein RB is a nuclear matrix junction protein and linked with DNA replication initiation site (72-74). Moreover, P53 and Rb were demonstrated to inhibit tumorigenesis by remodeling cytoskeleton (75, 76). 4) The common contradictory phenomenon observed in clinical tumor pathological sections is that, with the higher grade of malignancy, not only the percentage of proliferative tumor cells and multinucleated tumor giant cells but also the number of necrotic tumor cells increase(77-80). Our explanation is that rise of IOP drives tumor cells to divide, however, the division of mother tumor cell into two daughter tumor cells require the contraction force of M-phase actomyosin contraction ring should be greater than IOP (10, 17, 81, 82). The more malignant the tumor is, the more serious the function of actin is damaged (58-62, 64, 65, 83). Therefore, with the increase of tumor malignancy, the decrease of TLCM results in more tumor cells in the state of division, but on the other hand, decrease of contraction ring function don't overcome IOP and fail to divide mother tumor cell into two daughter tumor cells, resulting increasing number of multinucleated tumor giant cells or massive necrosis.

In conclusion, we believe that carcinogenesis is the result of uncontrolled IOP. Moreover, due to the alteration of Na^+/K^+ pump, cytoskeleton and nuclear matrix of malignant tumor cells, the morphological changes will affect

gene transcription through physical and mechanical mechanism, so the differentiation of malignant tumor is abnormal.

Targeted IOP regulatory molecules to treat malignant tumors

The new hypothesis have some predictions as following. 1) Tumor cells are more intolerant of hypotonic stress than normal cells. Although few people directly compare the tolerance between tumor cells and normal cells to hypotonic stress, the application of distilled water to lavage the chest and abdominal cavity after clinical operation can significantly reduce the spread and metastasis of malignancies and improve the survival rate of patients (84, 85). The survival time of mice inoculated with distilled water was significantly longer than that of normal saline (86). 2) Penicillin mainly inhibits the synthesis of cell wall, resulting in bacteria unable to withstand the IOP and death (13, 87). We think similar idea may also be used in the treatment of malignant tumors. Compared with normal cells, IOP of tumor cells increases faster and TLCM value is lower. Therefore, we think that if we target regulating molecules of IOP to further accelerate IOP and reduce TLCM value, it is possible to selectively kill tumor cells. As inhibition of Na^+/K^+ pump can dramatically increase IOP and inhibition of cytoskeleton can reduce TLCM, the combination of Na^+/K^+ pump and cytoskeleton inhibitors may have a stronger synergistic effect to kill tumor cells. At present, there are more than ten kinds of inhibitors of Na^+/K^+ pump and cytoskeleton respectively(88-96). In recent years, many anti-tumor drug screening experiments have proved that digoxin has obvious anti-tumor effect

(97-99), and many cytoskeleton inhibitors have been used in clinic for many years (100). Therefore, from these inhibitors, we may be able to screen effective drug combinations for cancer treatment. 3) molecules of IOP regulation may be a new target for cancer treatment.

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Author contribution: F.Z. had the idea for the article and performed the literature search and data analysis. F. Z., R.M.J. and C.Z. critically revised the work.

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