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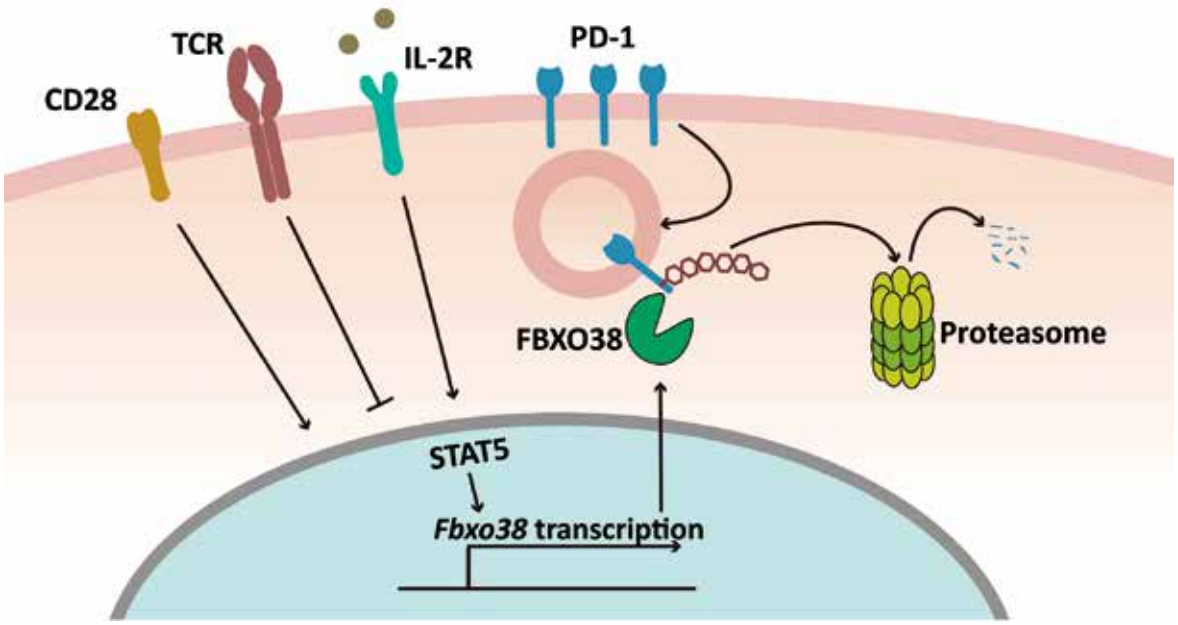
Regulation of "Checkpoint Molecule" Sheds New Light on Anti-Cancer Drug Development

By YAN Fusheng (Staff Reporter)

The rivalry between T cells and tumor cells somewhat mimics the scene of "*Tom and Jerry*," an animated series in which Tom (a house cat) rarely succeeds in catching Jerry (a mouse), mainly because of Jerry's cleverness and cunning abilities. In a way, tumor cells are like Jerry, in terms of their crafty and sneaky features. However, a recent finding by researchers at the Shanghai Institute of Biochemistry and Cell Biology (SIBCB), Chinese Academy of Sciences (CAS), might turn the plot in this cat-and-mouse game. The researchers discovered a new regulation pathway for the checkpoint molecules on T cells immunity, which points to a new direction for developing anti-cancer drugs.



A newly discovered PD-1 regulation pathway sheds lights on developing anti-cancer drugs. FBXO38, a protein that tags other proteins for proteasome degradation, was newly found to interact with PD-1, a braking molecule that restrains T cells immunity, and lead to its destruction. Thus, FBXO38 acts as a molecular switch, turning on/off the T cells immunity against tumor cells. An IL-2-mediated pathway has been identified to enhance FBXO38, which boosts T cells' immunity and hence offers clinical benefits. © Prof. XU Chenqi, SIBCB, CAS.

As a long-understood fact, tumor-infiltrating T cells usually become soft-hearted towards tumor cells, mainly because these T cells express large numbers of PD-1 on their surface, the well-known "molecular brakes" that restrain T cells activity when bound by other ligands, such as PD-L1. This offers a loophole for tumor cells. As notorious masters of opportunism, tumor cells express high levels of PD-L1 to hijack this pathway, inhibit T cells' activity and escape the latter's immune attack – in other words, tumor cells tread the brakes on T-cell anti-tumor immunity. But, how come the T cells are willing to show this weakness to their opponents, by showing large numbers of braking spots on their surface? This has long puzzled scientists.

This puzzle has been solved by a recent study, led by Prof. XU Chenqi at the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The scientists found that FBXO38, a protein that marks other proteins for degradation, interacts directly with the braking molecules PD-1 and therefore downregulates PD-1. However, the tumor-infiltrating T cells usually express low levels of FBXO38, which leads to the observed high levels of cell-surface PD-1 on activated T cells within tumors. This study was published November 29, 2018 in the high-impacting journal of *Nature*.

Towards New Biology of PD-1 Regulation

Scientists already knew that PD-1 is a braking spot for maintaining "self-tolerance" – preventing the activated T cells from attacking cells indiscriminately – and sneaky tumor cells express PD-L1 to tread on this braking spot to evade T cells' attack. Actually, blocking antibodies against the PD-1/PD-L1 pathway has yielded many blockbuster therapeutics against cancers, and relevant pioneering works have earned the 2018 Nobel Prize in Physiology or Medicine. Owing to its huge clinical significance, how PD-1 level is regulated at the transcriptional level, or the RNA level, has been extensively studied. Yet little has been known concerning PD-1 regulation at the protein level, which shall deepen our understanding on PD-1 regulation and may provide new target sites or strategies for therapeutic interferences. Hence, XU's lab sought to explore this unknown territory.

They firstly identified more than 100 proteins that specifically bound to the cytoplasmic domain of PD-1, in which two candidates, FBXO38 and FBXO47, stand out for their ubiquitination activity – marking other proteins for degradation. Reciprocal co-immunoprecipitation experiments, using either labeled FBXO38 or PD-1 to fish one another, both confirmed their physical interactions. Their functional interactions were then confirmed by: overexpression of *FBXO38*[1] led to lower amounts of PD-1 on the cell surface of activated T cells; whereas *FBXO38* gene-knockdown caused higher level of PD-1. However, the other candidate, FBXO47, was found to be functionally irrelevant. Thus, they inferred that FBXO38 regulates the PD-1 dynamics on the surface of activated T cells, and the FBXO38 level negatively correlates to the PD-1 level.

Next, they studied whether FBXO38 could directly mediate PD-1 ubiquitination, *i.e.*, mark PD-1 for proteasomes degradation, and thus lower the PD-1 level. Co-transfection of the two proteins, FBXO38 and PD-1, confirmed a FBXO38-dependent poly-ubiquitination of PD-1. Consistently, increased expression of *FBXO38* led to increased PD-1 poly-ubiquitination; and *vice versa*. Drawing on the mutagenesis data, they confirmed: Lys233 is the predominant ubiquitination site in PD-1; truncated FBXO38 by omitting the catalytic domain for ubiquitination, yields a reduced PD-1 ubiquitination. Moreover, the enzyme family that FBXO38 belongs to, usually catalyze Lys48-linked poly-ubiquitination on the target protein. Following this clue, they observed that mutation at the Lys48 site on ubiquitin completely abolished the effect of FBXO38-mediated ubiquitination and downregulation of PD-1.

Drawing on these converging data, they described a new regulation pathway for PD-1 at the protein level: FBXO38 mediates Lys48-linked poly-ubiquitination at the Lys233 site of PD-1, which regulates PD-1 level by proteasomes degradation.

Physiological Role of FBXO38

Then, they sought to further explore the physiological role of FBXO38 in mouse model and concluded that

[1] *FBXO38* indicates the human gene for FBXO38; while *Fbxo38* the mouse gene for FBXO38.

FBXO38 plays a key role in boosting the anti-tumor immunity of T cells mediated by downregulating the PD-1 level in activated T cells. This conclusion was drawn on compelling evidence. They firstly produced the conditional *Fbxo38*-knockout mice ($Fbxo38^{CKO}$ mice), in which the *Fbxo38* gene was specifically silenced in T cells. They found that the FBXO38 deficiency increases the cell-surface levels of PD-1 in activated T cells. In cancer models, they also found that $Fbxo38^{CKO}$ mice showed more aggressive tumor progression compared to wild-type mice. The FBXO38 deficiency led to increased PD-1 level and exhaustion features in T cells, hence more aggressive tumor progression. Notably, this nasty scenario can be rescued by anti-PD-1 treatment.

In addition, *FBXO38* transcription level of human T cells was found to be substantially lower within tumor microenvironment, compared with that in peripheral blood samples. This observation provides a plausible explanation for the puzzle concerning why tumor-infiltrating T cells tend to express high levels of PD-1 on cell surface and fail to kill tumors effectively.

Newly Found Role of IL-2

Consistently, *Fbxo38* transcription was also found to be lowered in tumour-infiltrating T cells in mice. This scenario can be reversed by supplementing IL-2, a type of cytokines that has been approved for clinical use against human cancers though its mechanism remains unclear.

By carefully examining mouse tumour-infiltrating T cells, they found that these T cells can receive antigen stimulation, but often lack sufficient co-stimulation and cytokine stimulation – auxiliary but indispensable pathways for producing an effective immune response. Thus, they examined many different cytokines to see whether their supplementations can turn the tide by upregulating *Fbxo38* transcription. Surprisingly, only IL-2 supplementation was found to have this effect and its unique feature was attributed to the direct binding of STAT5 – a major transcription factor activated by IL-2 – to the promoter of the *Fbxo38* gene. As widely expected, their finding – IL-2 upregulates FBXO38, downregulates PD-1 and subsequently boost the anti-tumor immunity of T cells – could be one of the mechanisms explaining its clinical benefit.

Implications

Summed up, this study discovers a new biology of PD-1 regulation at the protein level, in which FBXO38 marks PD-1 for proteasomes degradation, thus leads to reduced level of PD-1 and enhanced immunity strength of activated T cells in killing tumors. Moreover, they also found that IL-2 supplementation significantly upregulates FBXO38 level, which presents a possible explanation for the observed clinical benefits by IL-2 treatment in cancer patients. Both two findings highlight the potential clinical benefits by enhancing FBXO38 level in the tumor-infiltrating T cells. Strategies to target the newly found FBXO38/PD-1 regulation pathway could yield novel routes of cancer immunotherapy, which might present as a game changer in the rivalry between the immune system and the sneaky tumors – *This time, Tom may finally have its day and catch Jerry*.

Reference:

Xiangbo Meng, *et al*. FBXO38 mediates PD-1 ubiquitination and regulates anti-tumour immunity of T cells. *Nature*. Published online November 28, 2018. doi: 10.1038/s41586-018-0756-0.