

## Greatwall Kinase Oncogenic Properties Open New Horizons for Novel Human Cancer Therapies

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### Abstract

Protein phosphorylation ensures the accurate sequence of events underlying multiple signaling pathways, such as cell survival and cell proliferation. Using a combination of cell biology and molecular techniques, Vera et al. [20] have discovered an active role of Greatwall kinase in cell proliferation, transformation and invasiveness of human cancers through the Akt pathway, one of the most acknowledged oncogenic signaling networks in cells. This paper opens new horizons for human therapies in which Greatwall kinase can be used as a potential oncogenic marker and/or as a potential therapeutic target in aggressive human cancers.

**Keywords:** Greatwall kinase, growth, invasiveness, phosphorylation, phosphatase, proliferation

### Introduction

Cells are exposed to a plethora of extracellular signal, internalized and processed by complex signaling pathways and resulting in a specific biological response that is crucial for cell survival. It is largely known that most of the cell signaling pathways are tightly regulated by reversible post-translational modification events [1,2]. For instance, a delicate balance between protein phosphorylation and dephosphorylation is essential to ensure cell division and cell proliferation. The derangement of this equilibrium has been extensively linked to tumorigenesis and pathological disorders. Of note, most identified oncogenes are encoded by protein kinases and the majority of the tumor suppressors are phosphatases that counterbalance kinase activity. Therefore, deciphering how phosphorylation mechanisms orchestrate physiological processes such as cell division, migration and proliferation are essential not only for understanding human diseases but also for developing anti-cancer treatments and designing novel cancer therapies.

One of the most important oncogenic signaling cascades in which successive events of protein phosphorylation are highly relevant is the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR)-dependent pathway [3-6]. In this tremendous and tangled pathway, Akt is the most studied serine/threonine kinase because it acts as an epicenter of this network [7]. A blooming research work has shown that Akt plays a decisive role in cell survival, growth, migration, proliferation and cell cycle progression, determining cell fate mostly by regulating fundamental cell transcription factors [3,8-9]. Importantly, a constitutively activated form of Akt leads to cell cycle deregulation and uncontrolled cell proliferation, which are hallmarks of many human cancers. Hyperactivation of Akt has been found in cell models of renal, breast and prostate cancer [10-12].

In response to extracellular signals and growth factors, Akt is activated, reaching its maximal activity when phosphorylated on two residues: threonine 308 and serine 473 [3,13]. In spite of papers addressing a spectrum of kinases and

phosphatases governing the on-off switch for Akt activity such as glycogen synthase kinase-3 (GSK3), protein phosphatase 2A (PP2A), PH domain and leucine-rich repeat protein Phosphatases (PHLPP) (explained in the next section) have been emerged, not all pieces are placed in this intricate puzzle [14-19]. In a recent paper [20], outstanding findings underlying Akt and cell survival have been highlighted. Vera and colleagues have discovered a new pathway controlling 'GSK3-PHLPP-Akt' activities in cells in which the mitotic Greatwall (Gwl) kinase is critical [20]. This is the first study showing that Gwl could be a potential drug for human cancer therapies.

### Authors' Results

In 2004, the Gwl kinase was identified as a mitotic kinase in *Drosophila* [21,22]. Later on, in 2009, the Castro and Lorca group demonstrated that the classical activation of cyclin B-CDK is mediated by the regulation of the phosphatase PP2A in a complex with B55 regulatory subunit (PP2AB55), which is ultimately a target of Gwl in *Xenopus* egg extracts [23]. These observations were also validated in the mammalian Gwl homolog Microtubule Associated Serine Threonine Kinase Like (MASTL) [24,25].

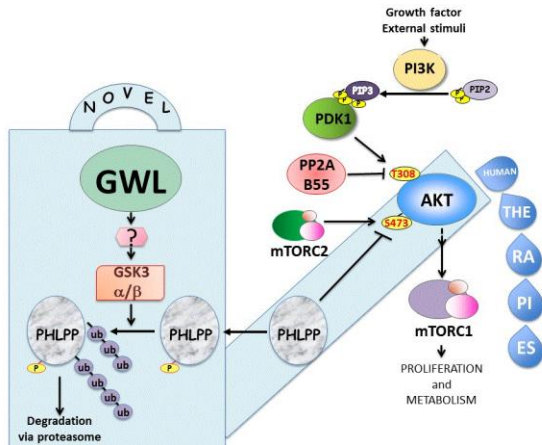
PP2A is one of the main serine-threonine phosphatases involved in controlling multiple cellular signaling pathways in mammalian cells [26,27]. PP2A is considered as a tumor suppressor [28-30]. It has been shown that PP2A promotes cell survival by negatively regulating different signaling networks such as the PI3K-Akt pathway [2]. Accordingly, loss of PP2A activity mediates oncogenesis by activating the PI3K-Akt routes [2].

Biochemical studies in *Xenopus* showed that PP2AB55 inhibition by Gwl protein kinase is not direct, rather mediated by its substrates: cyclic

adenosine monophosphate-regulated phosphoprotein 19 (Arpp19) and  $\alpha$ -Endosulfine (ENSA) [31,32]. Gwl-Arpp19/ENSA-PP2AB55 pathway is conserved through evolution as reported in *Drosophila*, budding yeast and plants [33-35]. Further, Vera et al. have reported that Gwl has an overpowering function during cell survival, proliferation and human malignancies through regulation of Akt [20]. Notably, they have found that overexpression of Gwl not only promotes cell proliferation, migration, invasion via its kinase activity in breast and colon cancer cells but also promotes transformation in primary human fibroblasts. In addition, overexpression of Gwl promotes tumor growth in a xenograft mouse model *in vivo* [20]. Using human phosphor-kinase array technology, they analyzed the phosphorylation of 43 different kinases involved in different oncogenic pathways upon Gwl overexpression or silencing. These experiments uncovered that Gwl overexpression triggered dephosphorylation of the inhibitory sites of the GSK3, promoting its activation, and subsequent increase of Akt phosphorylation in S473. In addition, the Gwl knockdown caused a decrease in Akt phosphorylation at S473. Further, they demonstrated that Akt inhibition is sufficient to revert the Gwl overexpression phenotypes in all the tested cell lines.

*In vitro* kinase assays indicated that Gwl kinase is not the direct kinase that phosphorylates Akt at S473. Then, the authors asked if Gwl modulates Akt indirectly through well-known regulators of Akt such mTOR2, [36] and PHLPP, [37]. Briefly summarizing, using different experiments, Vera et al. showed that neither decreased PP2AB55 activity through Arpp19/ENSA overexpression nor increased mTOR2 activity mediates oncogenic Gwl functions through Akt. They rather pointed out that Gwl-dependent increase of Akt phosphorylation levels is due to a drastic reduction in PHLPP, the phosphatase responsible for Akt dephosphorylation at S473 (Figure). It is worth mentioning that PHLPP is degraded by the proteasome upon phosphorylation by GSK3, a kinase that the authors found highly activated upon Gwl overexpression. This lead them to investigate if Gwl could have an impact on GSK3

activity and therefore on PHLPP levels and Akt dephosphorylation at S473. Indeed, this seems to be the case. Thus, Vera et al. results demonstrate that Gwl hits PHLPP levels through GSK3 activity and consequently regulates Akt-phosphorylation status.



**Figure. Simple schematics of the novel Greatwall functions in PIP3-Akt-mTORC1 pathway.** On the right, classical Akt (shown in blue) regulation through protein kinases (PDK1 and mTORC2, depicted in dark green) and phosphatases (PP2A, in pink; PHLPP in grey). On the left, Akt regulation through Greatwall kinase (GWL, in light green). The novel pathway is represented as a watering can depicted in light blue that drops 'potential' human therapies. Courtesy of J. Vera (modified by MA Juanes)

In conclusion, Vera et al. paper revealed a new substrate of Gwl kinase and a new pathway 'GSK3-PHLPP-Akt' in which the Gwl kinase contributes to cell transformation and invasion. Because of the implications of this pathway in cell survival and carcinogenesis, this is an outstanding breakthrough since Gwl could foster novel cancer therapies. For example, drugs that inhibit Gwl could be used as a new therapeutic target.

One important issue that remains unsolved for the future is how Gwl exactly controls GSK3 activity. Challenging future research will

contribute to better understanding of the fascinating mechanisms underlying Gwl regulation that facilitates proliferation and oncogenic processes through Akt and GSK3. The excellent and distinguished research by Vera in Castro and Lorca laboratory about Gwl–Akt signaling pathway in human malignancies highlights the tight phospho-regulatory mechanisms that govern signaling pathways inside the cells for successful cell growth and survival.

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