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Hsp90α/β associates with the GSK3β/axin1/phospho-β-catenin complex in the human MCF-7 epithelial breast cancer model

Leanne C. Cooper [a], Earl Prinsloo [a,*], Adrienne L. Edkins [a], Gregory L. Blatch [a,b]

[a] Biomedical Biotechnology Research Unit, Department of Biochemistry, Microbiology and Biotechnology, P.O. Box 94, Rhodes University, Grahamstown 6140, South Africa
[b] School of Biomedical and Health Sciences, Faculty of Health, Engineering and Science, P.O. Box 14428, Victoria University, Melbourne, Vic 8001, Australia

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ABSTRACT

Hsp90α/β, the signal transduction chaperone, maintains intracellular communication in normal, stem, and cancer cells. The well characterised association of Hsp90α/β with its client kinases form the framework of multiple signalling networks. GSK3β, a known Hsp90α/β client, mediates β-catenin phosphorylation as part of a cytoplasmic destruction complex which targets phospho-β-catenin to the 26S proteasome. The canonical Wnt/β-catenin pathway promotes stem cell self-renewal as well as oncogenesis. The degree of Hsp90α/β involvement in Wnt/β-catenin signalling needs clarification. Here, we describe the association of Hsp90α/β with GSK3β, β-catenin, phospho-β-catenin and the molecular scaffold, axin1, in the human MCF-7 epithelial breast cancer cell model using selective inhibition of Hsp90α/β, confocal laser scanning microscopy and immunoprecipitation. Our findings suggest that Hsp90α/β modulates the phosphorylation of β-catenin by interaction in common complex with GSK3β/axin1/β-catenin.

1. Introduction

The canonical Wnt/β-catenin signalling pathway bridges the narrow divide between stem cells and cancer [1,2]. Wnt stimulation (exogenous or autocrine) kickstarts a series of events enabling the nuclear accumulation of β-catenin and the transcription of known oncogenes like *c-jun*, *c-myc* and *cyclin D1* (reviewed by Macdonald et al. [3]). Normally, tight regulation via a destruction complex comprising axin1, adenomatous polyposis coli protein (APC), glycogen synthase kinase 3β (GSK3β), protein phosphatase 2A (PP2A) and casein kinase 1-alpha (CK1α) maintains cytoplasmic β-catenin levels by phosphorylation of β-catenin resulting in its ubiquitination and targeting to the 26S proteasome [4–7]. Canonically, Wnt stimulation of the LRP5/6/Fz (Low density lipoprotein receptor-related protein 5/6 and Frizzled, respectively) complex triggers the disruption of the destruction complex by phosphorylation of LRP5/6 by CK1γ and GSK3β which results in the scaffolding protein, axin1, being sequestered away from the destruction complex to the membrane and subsequently degraded, thereby inhibiting β-catenin phosphorylation [7–9]. Recently, it has been shown that the presence of Wnt triggers the spacial sequestration of GSK3β, without apparent decrease in total protein levels, in multivesicular bodies allowing β-catenin nuclear translocation and subsequent complexation with T cell specific factor (TCF)/lymphoid enhancer binding factor 1 (LEF-1) transcription factor [10].

In cancer, the molecular chaperone heat shock protein 90 (Hsp90α/β) is involved in the proteostatic maintenance of oncoproteins [11]. This is in contrast to the role Hsp90α/β plays in maintaining signal transduction in normal cells by either interacting directly with transcription factors or modulating the kinases that regulate function by phosphorylation [12,13]. Previous reports have pointed to GSK3β as a Hsp90α/β client protein including: the requirement of Hsp90 in the autophosphorylation and maturation of GSK3β in rabbit reticulocyte lysates [14]; maintenance of stability and function of GSK3β in simian COS-7 and rat primary neuronal cultures [15] and the association of Hsp90 with mature GSK3β in human Hep3B hepatocellular carcinoma [16]. These studies have highlighted the potential interaction between Hsp90α/β and GSK3β and have shown that directed inhibition of Hsp90α/β decreases GSK3β steady state protein levels which in turn decreases β-catenin phosphorylation. Kurashina et al. [17] have argued against this model in adult T cell leukaemia/lymphoma (ATL) cells and claim that Hsp90α/β inhibition results in Akt (a Hsp90α/β client serine-threonine kinase) inactivation which prevents GSK3β inactivation and leads to β-catenin phosphorylation. Regardless, both systems are equally valid for the cell systems used in the studies described above. However, the role of Hsp90α/β in β-catenin metabolism is complex and not fully understood. In particular, the role of Hsp90α/β in the modulation of β-catenin and phospho-β-catenin levels needs investigation. Aberrant autocrine activation of the Wnt pathway has previously been reported in breast cancer [18].

Abbreviations: Hsp90α/β, heat shock protein 90; GSK3β, glycogen synthase kinase 3β.

* Corresponding author. Fax: +27 46 622 3984.
E-mail address: e.prinsloo@ru.ac.za (E. Prinsloo).

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