Highly Specific Antibodies Against Phosphorylated Ser125-NPM1

International Patent Application.
PCT/IB2011/052734

Available For Licensing/Technology Trnafer

IIPRD

INSTITUTE OF INTELLECTUAL PROPERTY
RESEARCH & DEVELOPMENT

About NPM120

- NPM1 (Nucleophosmin/B23) is a highly dynamic, nucleolar protein which plays important roles in diverse cellular processes such as ribosome biogenesis, centrosome duplication, maintenance of genomic stability and embryonic development
- NPM1 is a crucial gene to consider in the context of the genetics and biology of cancer. NPM1 is frequently overexpressed, mutated, rearranged and deleted in human cancer.
- It was shown to be involved in mitotic spindle formation and regulation of microtubule spindle tension
- Phosphorylation of NPM1 is likely to control aspects of function, localization and oligomerisation and could be related to nucleolar function and progression through mitosis

Inventors' Technology

• Phosphorylation of NPM1 at Serine 125 residue by Aurora Kinase A and/or B by a method of incubating NPM1 with Aurora Kinase

• Highly Specific Polyclonal Antibodies generated against Phosphorylated Ser125-NPM1

• Compositions comprising the Antibodies optionally along with pharmaceutically acceptable excipients

• Method for identifying phosphorylated NPMl by Aurora Kinase

• Method of detecting cancer, by raising and binding of highly specific antibodies against phosphorylated Ser125-NPMl.

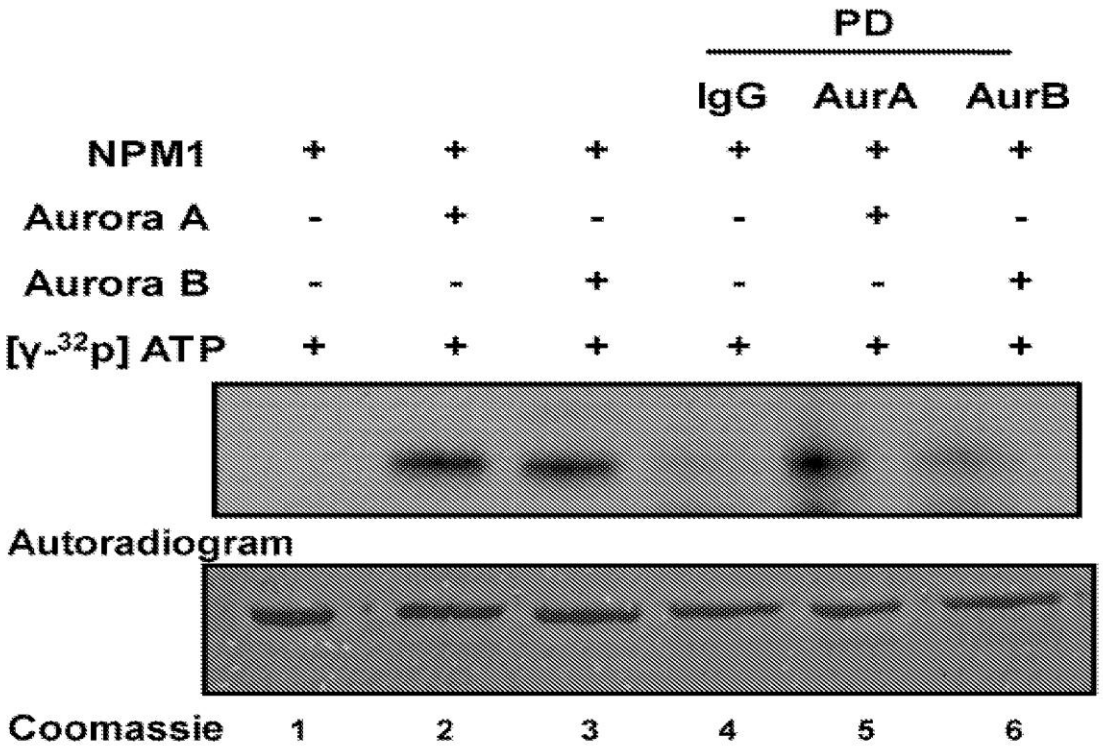
Novel Finding:

The inventors found that NPMl is a novel substrate of Aurora kinases A and B where both kinases phosphorylate at the same site, Ser125.

Studies Evidencing Inventors' Findings

• **Incubation of Aurora A and B complexes with NPM1 in presence of [γ32-ρ] ATP** showed that NPMl could be phosphorylated by both Aurora kinase A as well as -B containing complexes (lanes 5 and 6 of the below Figure). In vitro kinase assay of NPMl with recombinant Aurora A and -B was taken as positive control (Fig. D, lanes 2 and 3)

D



- **Deletion Mapping Analysis**

• In vitro kinase assay using Aurora A and -B with different C-terminal deletion mutants of NPMl \

• Fine-mapping of the probable region of Aurora kinase-mediated phosphorylation was done by making use of internal deletion mutants of NPM1 (del 120-126) and (del 120-132).

•Since the mutants lack only a single serine residue, Serl25 in the deleted stretch, with all possibilities, it could be the probable site targeted by Aurora kinases.

• **Aurora kinase-dependent in vitro kinase assay** results showed that mutation of Serl25 to either alanine or glutamic acid led to an almost complete abolishment of NPM1 phosphorylation (Fig. 2D below, lanes 6-7 and lanes 14-15, respectively). These data indicate that indeed Aurora kinases A and -B phosphorylate NPM1 at a single residue Serl25 in vitro.

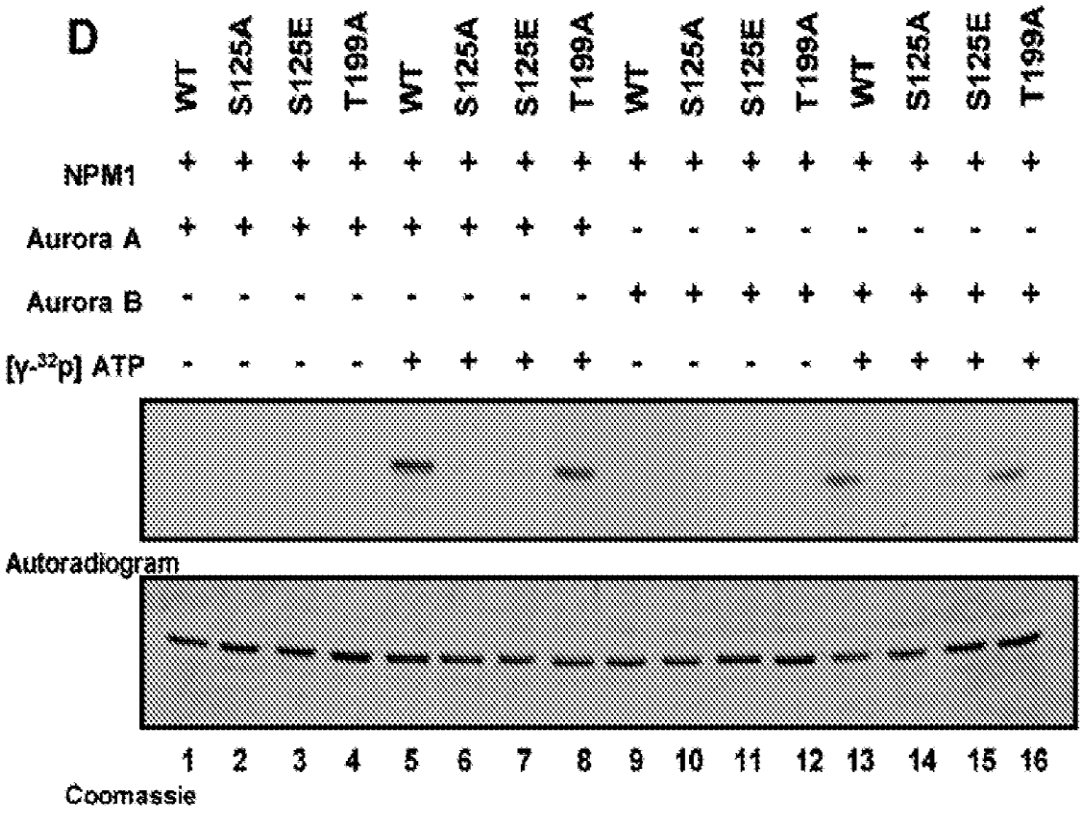
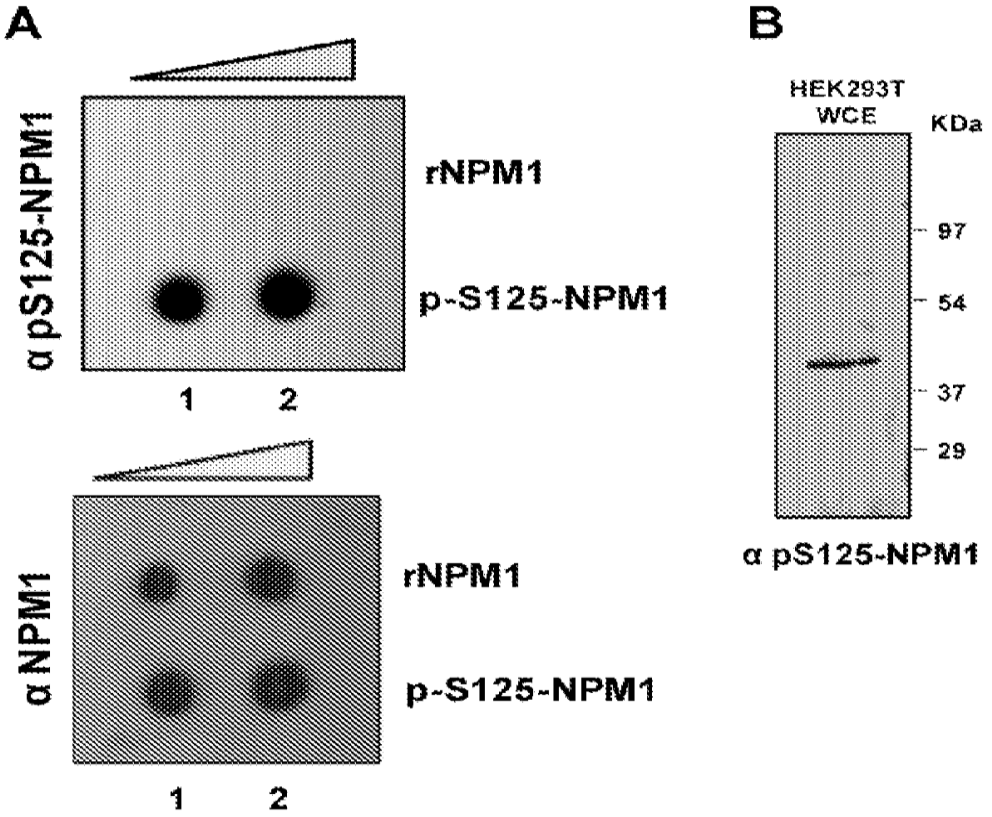


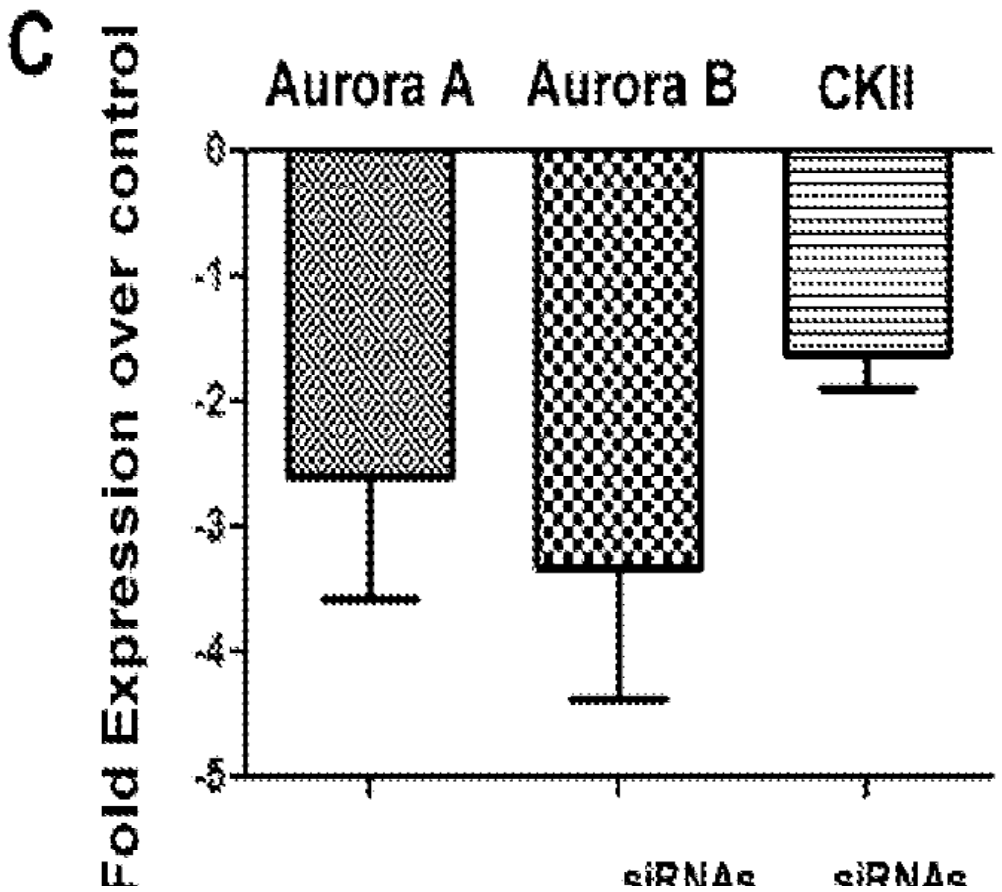
Figure 2

• In order to study the **subcellular distribution of pS125-NPMl**, polyclonal antibodies were raised in rabbit using KLH-conjugated NPMl -peptide phosphorylated at Serl25.

•The anti-pS125-NPMl antibody could recognize only the phosphorylated peptide and not the full-length recombinant WT NPM1 protein (Fig. A below). It also recognized a specific band in the whole cell extracts prepared from HEK293T cells (Fig. B below).



• In order to further establish that Aurora A and -B kinases phosphorylate NPM1 in the cells, the inventors resorted to **siRNA mediated knockdown of CKII, Aurora A and Aurora B in different combinations**. The knockdown efficiency of siR As against CKII, Aurora A and Aurora B over the scrambled R A control transfection was quantified in real-time RT-PCR analysis 48 hours post- transfection in HEK293T cells (Fig. C below)



• **Western blotting analysis** was performed in the silenced cells to see the effect on the phosphorylation of S125NPM1.

✓ These results show that Aurora A and -B are the bona fide kinases for NPM1.

• **Co-immunofiuorescence analysis** was performed to study the cell cycle-stage-specific association of pS 125 -NPMl with Aurora A as well as Aurora B.

✓ These results show that indeed pS125-NPMl associates mainly with Aurora B kinase during cytokinesis and could have an essential role to regulate the completion of cell division.

✓ This observation assigns a novel role for NPM1 in mitosis besides its known function in regulation of centrosome duplication.

• To directly assay the **role of p-S125NPMl in centrosome duplication**, FLAG-tagged (SI 25 A) and (S125E) mutants of NPMl were compared with FLAG-WT NPMl in their cellular functions.

✓ It was observed by the inventors that **overexpression of (SI 25 A) NPM1 resulted in centrosome amplification**. This observation of multiple centrosomes could be a consequence of deregulated centrosome duplication or an indirect effect of cytokinesis failure.

• Thus the present inventors disclose that t**he combinatorial effect of overexpression of Aurora kinases as well as their substrate NPMl may play an important role in facilitating cancer progression and hence could be a potential target for anti-tumor therapeutic interventions.**

• The inventors thus finally disclose that the **highly specific antibodies which are against the phosphorylated S125-NPM1 along with pharmaceutically acceptable carrier(s) could be used in the treatment of Cancer.**

Institute Of Intellectual Property Research & Development (IIPRD)

IIPRD

New Delhi Office
IFAIA Centre, S/20-22, Greater Noida Shopping Plaza
UPSIDC Site – IV, Greater Noida – 201 308, UP, India.

Pune Office:
D-1/4, HDFC Colony, Chinchwad,
Mumbai-Pune Road, Pune, India.
Contact Person: Tarun Khurana
Contact No.: +91-120-2342010-11

US Office
Suite 108G, 2000 Walnut Ave
Fremont CA 94538

E-Mail: iiprd@iiprd.com, info@khuranaandkhurana.com
Website: www.iiprd.com | www.khuranaandkhurana.com