Identification of a Nucleolar Protein, p130, As a Specific Binder to Doxorubicin by Chemical Genomics Approach

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Chemical genomics approach is one the functional genomics that can facilitate the identification of potential drug targets that interact with bioactive compounds or therapeutic drugs. We have used biopanning method with phage displayed cDNA library to find proteins that specifically bind to an anticancer drug.

Doxorubicin is a widely used anti-cancer drug that has cytotoxic activity against various types of cancer cells. Due to the four-ring structure in doxorubicin (Fig. 1), it assumed to intercalate between the base pairs of chromosomal DNA and prevent DNA replication or transcription. The DNA binding property of doxorubicin appeared to be critical for its anticancer activity, however, there were reports that doxorubicin might interact cellular components other than DNA such as RNA polymerase II or membrane components. Since the compound is widely used in chemotherapy of cancer patients, it is critical to identify the cellular components that interact specifically with doxorubicin to improve the anti-cancer activity as well as to reduce its side effects.

To examine the potential target protein against doxorubicin, we have used a biopanning method with a T7 phage library expressing human liver cDNA on the surface of phage. The phage library was screened against the immobilized doxorubicin (Fig. 1), and a phage clone was isolated. Sequence analysis showed that the cloned phage displayed the C-terminal region of p130 (or hNopp140). Previously, Nopp140 was identified as a nucleolus protein that has an important role in the assembly and transport of preribosome particle. It is one of the most highly phosphorylated proteins and has ATPase/GTPase activity. RNA polymerase I, transcription factor such as C/EBP, and casein kinase II were known to physically interact with hNopp140. Although the molecular and cellular function of hNopp140 was not completely revealed, it is assumed to have a critical function in the biogenesis of nucleolus as well as cell division

The C terminal region of hNopp140 that can bind to doxorubicin was further expressed in *E. coli* and purified. The recombinant protein could be phosphorylated by casein kinase II and oligomerized in the presence of

magnesium and fluoride ions as *in vivo* state. When the dissociation constant of the recombinant hNopp140 to immobilized doxorubicin was examined using BIAcore, it measured as 4.5 10 6M (Fig. 2). In contrast, DNA fragment failed to bind immobilized doxorubicin, indicating that hNopp140 interact with the ring structure of doxorubicin more specifically than DNA. Interestingly, doxorubincin bound to only the un-phosphorylated rather than phosphorylated form. The significance of the interaction between doxorubicin and hNopp140 with relation to the cytotoxic activity of doxorubicin was discussed.

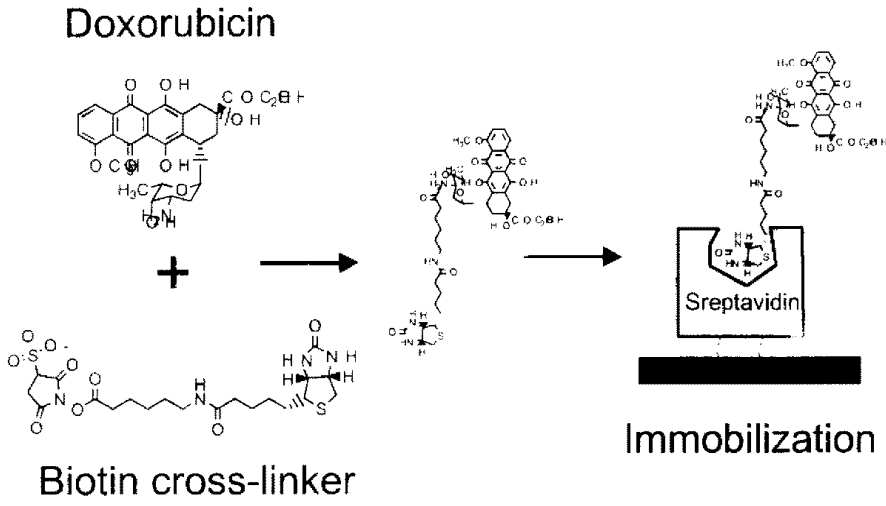

Fig.1

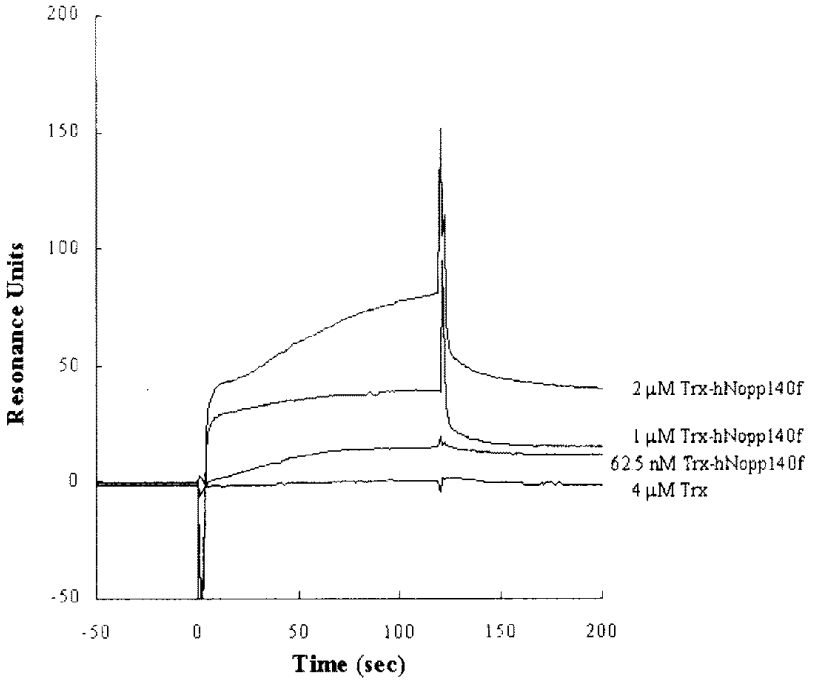

Fig. 2