The Interaction and Role of Nischarin and Cortactin within Invasive Cancers

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Introduction

Nischarin is a novel protein that was discovered by Suresh Alahari, PhD and his lab in 2000. The protein is located on chromosome 3p21 and is thought to behave as a tumor supressor in the metastasis process of several types of invasive cancers. Specifically, overexpression of the protein has been shown to inhibit breast tumor growth and metastasis [1]. Cortactin is a protein that localizes to cortical actin structures and plays a role in the regulation of the cytoskeleton and in certain aggressive cancers. Cortactin also has a significant role in the development and maturation of invadopodia, which is involved in the epithelial to mesenchymal transition (EMT) seen in invasive cancer progression [2]. My study examined the potential interaction these proteins have with one another and developed the groundwork for evaluating the regulation process they possibly undergo in cancer progression.

Hypothesis

We hypothesized, on the basis of previous research regarding the nature of Nischarin and cortactin, that the two proteins are involved in regulating one another in the progression of many invasive cancers.

Methods

COS-7 eukaryotic cells derived from monkey kidney were used as a stable cell line to examine the potential interaction of Nischarin and cortactin. After splitting the cells on to separate plates, the line was initially transfected with Myc-Beta-Galactosidase, Myc-Nischarin, and PM Cherry Cortactin. After transfection, cell lysis, and a BSA assay to determine protein concentration, SDS-PAGE was run. Using the SDS-PAGE gel, a transfer was completed to a Millipore© PVDF membrane, which was used to perform a western blot. This western blot was then developed to determine the presence of these proteins within the cell line. Once completed, GFP-Nischarin and GFP-Nischarin + cortactin were also transfected to determine whether there was indication of interaction between Nischarin and cortactin. Immunofluorescent imaging was used to visualize the GFP (green fluorescent protein) and PM cherry tags that were attached to Nischarin and cortactin respectively in order to see whether the proteins were expressed within the same cells. A technique called co-immunoprecipitation was then run to further determine whether the two proteins were indeed interacting. Western blotting was also performed on these additional samples.

Study Objectives

- Elucidate a better understanding of the potential interaction between Nischarin and cortactin in the progression of cancer.
- Use COS-7 cell line (derived from African green monkey kidney) as a model to examine this interaction between the two proteins.
- Discovering the presence of this interaction will assist in understanding not only the pathway of certain invasive cancers, but also possible treatment options depending on the details of their regulation of one another.

Results

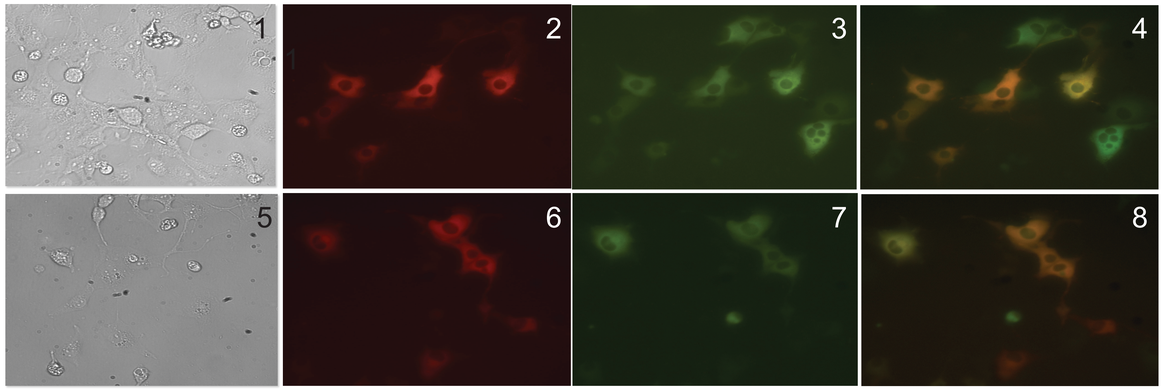

Figure 1-8. GFP-Nischarin + cortactin transfection (left to right): phase contrast (1,5), immunofluorescent images of PM cherry cortactin (2,6),GFP-Nisch (3,7), and GFP-Nischarin + cortactin transfection (4,8)

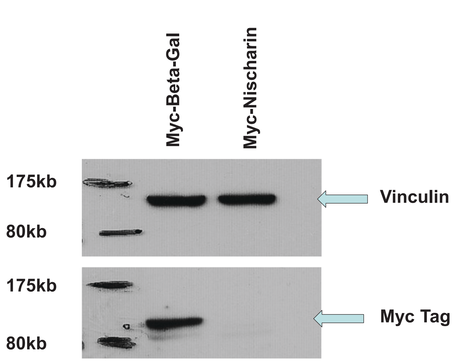

Figure 9. Western blots with Kodak X-OMAT 2000 Processor

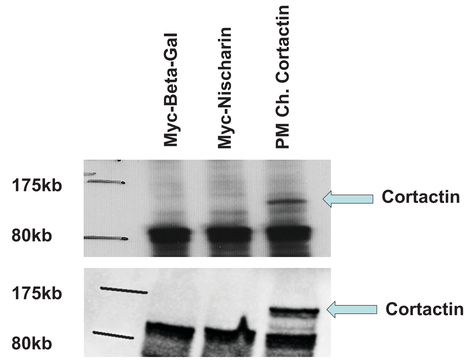

Figure 10. Western blots, Top: Kodak X-OMAT Processor Bottom: Bio-Rad Gel Doc XR System

Summary

- Cortactin and Nischarin are both expressed in the COS-7 cell line.
- GFP-Nischarin and PM cherry cortactin can be successfully transfected into the COS-7 cell line together and are readily visualized.
- Cortactin and Nischarin can be transfected in to the same cell, as shown with the immunofluorescence, which indicates the possibility of interaction between the two proteins.

Future Studies

- Further evaluate the presence of an interaction between Nischarin and cortactin via co-immunoprecipitation.
- Examine the effects of cortactin expression on Nischarin expression and vice-versa via manipulation of expression levels.
- Determine whether there is potential for aggressive cancer treatment through regulation of one or both of the proteins expression levels.

References

1.Jain P., Baranwal S., Dong S., Struckhoff A.P., Worthylake R.A., Alahari S.K. (2013). Integrin-binding protein nischarin interacts with tumor suppressor liver kinase B1 (LKB1) to regulate cell migration of breast epithelial cells. J. Biol. Chem. 288, 15495-15509.

2.MacGrath S.M., Koleske A.J. (2012). Cortactin in cell migration at a glance. J. Cell Sci. 125 (7), 1621-1626.

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