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TITLE: NOS2 regulation in NPC
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PROJECT DESCRIPTION

Cholesterol processing abnormalities are well characterized in Niemann-Pick type C (NPC) disease, however, little is known about the subsequent cell signaling consequences of impaired cholesterol transport. The goal of this application is to understand the cellular responses to lost NPC1 function. We have recently identified that NPC fibroblasts exhibit nearly identical cell signaling alterations as observed in cystic fibrosis (CF) epithelial cells including reduced expression of the inducible form of nitric oxide synthase (NOS2) compared to wt fibroblasts upon challenge with inflammatory cytokines, suggesting alterations in cell signaling cascades. Other similarities at the cellular level between NPC and CF include accumulation of free cholesterol, over expression of the small GTPase RhoA and increased protein levels of the interferon-g (IFN-g) signaling protein signal transducer and activation of transcription-1 (STAT1). The hypothesis of this study is that impaired cholesterol processing due to reduced or lost NPC1 function leads to aberrations in NOS2 regulation through RhoA and STAT1-dependent mechansims. The identification of these cell signaling differences in NPC represents an avenue to begin exploring the impact of impaired cholesterol transport on intracellular functions and to clarify potential pathologic processes. For example, RhoA and STAT1 signaling are key to regulating inflammatory responses, RhoA is an important modulator of cell proliferation and dendritogenesis (a process improperly regulated in NPC), and increased RhoA activity has been associated with increased Tau phosphorylation. Also, nitric oxide (NO) is an important regulator of inflammation and neuronal function. Improved understanding of these signaling alterations could identify new sights of therapeutic intervention, as well as provide better markers of disease severity or treatment efficacy. To pursue our goal of understanding RhoA, STAT1, and NOS2 regulation in NPC cells, the following specific aims will be pursued:

Aim 1. To examine RhoA expression, modification, and activation in NPC models.
Aim 2. To examine STAT1 signaling regulation in NPC models.
Aim 3. To determine the role of ceramide-mediated signaling in NOS2 regulation in Niemann-Pick disease.

The successful completion of this study will provide a foundation of information about the intracellular impact of lost NPC1 function in fibroblasts that can be extended in the future to in vivo models of NPC and how these pathways affect neurological function and pathological processes. How these pathways are influenced in other forms of Niemann-Pick disease will also be examined.

FINAL STATUS REPORT
Dated 4/30/2006

The goal of this project was to examine cell-signaling regulation in NPC cells based on initial findings that nitric oxide synthase-2 (NOS2) expression is reduced in NPC. Early data suggested a possible role for the small GTPase RhoA in NPC-related signaling alterations. Rho family GTPases are signaling proteins that control a number of cellular functions, many relevant to NPC. The hypothesis developed during these studies was that altered GTPase signaling contributes to many aspects of NPC pathophysiology.

To begin addressing the potential role of RhoA signaling in NPC, the basal activation state of RhoA and Ras GTPases were examined. Small GTPase activity is determined by the amount of GTPase in the GTP-bound form. The relative content of RhoA-GTP and Ras-GTP was determined in wt and NPC human fibroblasts normalized to total RhoA or Ras content. Both RhoA and Ras exhibited increased GTP binding in NPC fibroblasts compared to wt fibroblasts indicating a basal increase in GTPase activation as a result of lost NPC1 function. These data confirm that RhoA and Ras GTPases are strong candidates for effectors of NPC-related signaling alterations.

A prediction of elevated GTPase signaling, particularly RhoA and Ras, is a skewing of cell-signaling regulation to favor proliferation. For example, a study by Liberto et al demonstrated that elevated Ras and RhoA signaling in breast cancer cells resulted in reduced p21/waf1 and elevated cyclin D protein expression. These signaling parameters were examined in NPC fibroblasts to test whether they would exhibit these changes consistent with elevated GTPase activation. The cyclin kinase inhibitor p21/waf1 exhibits decreased expression in NPC fibroblasts compared to wt controls. This result is consistent with an elevation of RhoA signaling. Correspondingly, expression of cyclin D is significantly elevated in NPC fibroblasts compared to controls. Immunostaining for cyclin D also reveals increased expression in NPC fibroblasts. These data demonstrate a significant alteration in proliferation control in NPC fibroblasts. An hypothesis exists that improper entry into a proliferative cycle triggers neuronal death in Alzheimer's disease. Our future goals are to explore proliferation signaling in NPC neuronal cells as a potential mechanism of neurological decline.

Another reported consequence of NPC is impaired neurite outgrowth in embryonic neuronal cells isolated from Npc1 -/- mice (2). To determine if this is likely due to cholesterol accumulation, PC12 cells were treated with the cholesterol transport inhibitor U18666a (5 μg/ml) for 24 hours prior to the addition of NGF. We know in epithelial cells and fibroblasts that U18666a treatment results in elevated RhoA signaling (data not shown). U18666a effectively prevented neurite outgrowth in PC12 cells and the addition of the geranylgeranyl transferase inhibitor GGTI-286 partially restored neurite outgrowth in the presence of U18666a. These data demonstrate that inhibition of cholesterol transport mimics the NPC phenotype of inhibited neurite outgrowth and that geranylgeranyl-modified RhoA is a strong candidate to mediate this effect.

The above data demonstrate that GTPase signaling is elevated in NPC fibroblasts and relevant to a number of NPC-associated phenotypes. Elevated RhoA and Ras signaling may be central to the pathogenic consequences of impaired cholesterol transport in NPC. If true, dissection of these signaling interactions in NPC will reveal a set of new potential targets for therapeutic intervention. This centrality

suggests that new therapies aimed at small GTPase signaling has the potential of preventing or at least slowing neurological degeneration associated with NPC.

PUBLICATIONS:

No Publications on this Work To Date