Identification of a Viral Protein that Inhibits Insulin Receptor Activation

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1 Overview

• tandem affinity purifications using viral protein "bait"

• identification of a viral protein that binds insulin receptor (INSR) and insulin like growth factor 1 receptor (IGF1R)

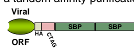
• determine if viral protein activates or inactivates receptors

2 Introduction

Viral genomes encode proteins that interact with host proteins, where natural selection drives the propagation of those interactions that impart some sort of selective advantage. For example, viral-host protein interactions may result in enhanced proliferation (and therefore enhanced viral particle replication) or subversion of the host immune system. It is the latter that provides the impetus for our systematic study of viral-host protein interactions via tandem affinity LC/MSMS. Using viral proteins as baits, these studies can rapidly provide lists of host-viral interactions; however, the biological effects of these interactions remain unknown, requiring further experimentation. Follow up studies of one component of this interactome is described here. A viral protein from Rhesus cytomegalovirus (CMV) was found that binds to **insulin receptor (INSR)** and/or **insulin-like growth factor 1 receptor (IGF1R).** In this example, commercial phospho-specific antibodies were available to test whether the viral protein could activate or inactivate either receptor. Homologs (from human CMV and a different monkey CMV open reading frame) of the Rhesus viral protein were also tested for their ability to bind INSR or IGF1R.

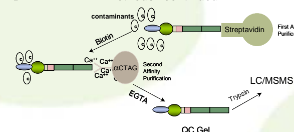
3 Methods

Bioinformatic tools were used to derive a list of putative extracellular (membrane or secreted) viral proteins of unknown function. These proteins were picked for gene synthesis with a tandem affinity purification tag:



Streptavidin beads were used to collect the tagged viral protein from conditioned media, and the beads (plus bait) were mixed with various mammalian cell cultures and primary cell lysates. After washing, the selective binding partners were eluted from the beads, digested with trypsin, and analyzed by LCMSMS (LTQ).

4 Methods –continued-



Flow cytometry was used to confirm the binding of the viral factor to cell surfaces.

Western blots were performed using anti-phospho-INSR and anti-phospho-IGF1R as primary antibodies.

5 Results

A cytomegalovirus that infects Rhesus monkeys codes for a Type 1 membrane protein called rh27 that was shown to be non-essential for *in vitro* viral replication (and therefore a gene that might be important for *in vivo* replication and virulence). The extracellular portion of this protein was expressed with a C-terminal affinity tag, which was used to find binding partners from a series of human cell culture lysates (T cells, B cells, NK cells and monocytes). The bound proteins were identified by LC/MSMS followed by a database search. Figure 1 shows two of many MSMS spectra assigned to INSR and IGF1R, and Fig. 2 demonstrates that the coverage for these proteins was 30% and 12%, respectively. We have never observed these receptors as background contaminants in previous affinity experiments, and they are presumed to interact specifically with this viral protein. To confirm that this viral protein binds to cell surfaces, flow cytometric analysis was performed (Fig. 3).

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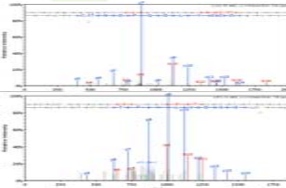

Figure 1: (top) MSMS spectrum of $(M+2H)^{+2}$ = m/z 1007.2 from rh27 pull-down identified as FVMDGGYLDQPDNCPER from INSR. (bottom) MSMS spectrum of (M+2H)+2 = m/z 936.6 from rh27 pull-down identified as ASCESDVLHFTSTTTSK from IGF1R.

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Figure 2: Standard capillary LC/MSMS approach using an LTQ with an X!Tandem database search identified both insulin receptor (INSR) and insulin-like growth factor receptor (IGF1R). Regions in yellow indicate identified peptides.

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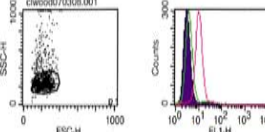

Figure 3: Flow cytometry demonstrating that the viral protein bait binds to a monocyte cell line. The left panel indicates the gating that was used. The affinity tag contains a hemagglutinin (HA) epitope. For the right panel, purple shows unlabeled cells, green is the anti-HA antibody alone, and pink demonstrates the shift that occurs with the tagged viral protein, plus anti-HA.

9 Results -continued-

These receptors are tyrosine kinases that autophosphorylate upon binding to their ligands, insulin and insulin-like growth factor 1. From this observed binding event we hypothesized that the viral protein serves as an activating ligand, since activation of IGF1R would put the host cell in a proliferative state, a condition that would seem to be useful to a virus trying to replicate itself. However, using phospho-specific anti-INSR antibodies and western blotting, we show that the viral protein does *not* induce autophosphorylation, but instead appears to inhibit it (Fig. 4). Using an identical approach for IGF1R, the viral protein had no effect, stimulatory or inhibitory (Fig. 5).

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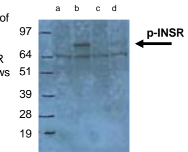

Figure 4: Inhibition of ligand-induced autophosphorylation of INSR in monomac 6 (MM6) cells. Western blot used anti-phospho-pY(1150/1151) INSR as the primary. Lane (a) shows cell lysates from unstimulated cells. Lane (b) is the positive control of 10 minute insulin stimulation. Lane (c) is viral protein alone, showing no receptor activation. Lane (d) shows that pre-treatment of cells with the viral protein for 30 minutes prior to insulin inhibits activation.

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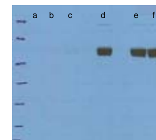

Figure 5: Undetectable activity in 293T cells. Western blot using anti-pY980 IGF1R. Lane (a) shows cell lysates from unstimulated cells. Lanes (b) and (c) are from viral protein alone (with two different affinity tags). Lane (d) is IGF1 ligand stimulation. Lanes (e) and (f) show 10 and 30 minute pre-incubation with viral protein prior to IGF1 stimulation.

12 Conclusions

• monkey herpes virus (Rhesus cytomegalovirus) encodes a membrane protein whose extracellular region binds to insulin receptor and insulin-like growth factor 1 receptor

• tagged soluble extracellular viral protein does not activate insulin receptor, but instead seems to inhibit activation by insulin

• the viral protein does not activate nor does it inhibit activation of insulin-like growth factor 1 receptor