Proteomics-Based Identification of Estrogen-Responsive Biomarkers in the Seminal Vesicle: Development of a Bioassay for Screening Estrogenic Compounds in Males

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Introduction

Exposure to exogenous estrogens in males can induce various health complications, including cancers and reproductive and metabolic disorders. Uterotrophic assays are traditionally used for screening estrogenic compounds by measuring changes in the wet weight of the uterus. However, with **no established male analog**, these studies are solely conducted in female rodent models. Previous studies report that the **seminal vesicles (SV) enlarge after exposure to estradiol** in orchiectomized (ORDX) mice. However, assessing estrogenicity solely based on the wet weight of the SV is limited as weakly estrogenic compounds may escape detection. Therefore, protein-based markers are beneficial to complement the weight-based assessments. **In this study, we report a discovery-driven proteomic analysis of 17β-estradiol's (E2) effects on the SV for the first time to identify potential biomarkers for screening estrogenic compounds in males.**

Results

Figure 2. Wet weight of the excised SVs.

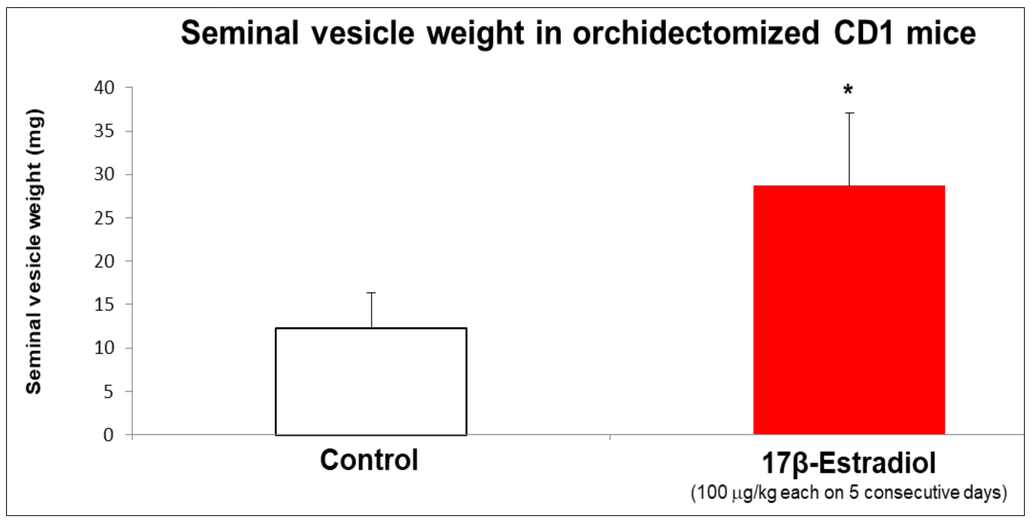
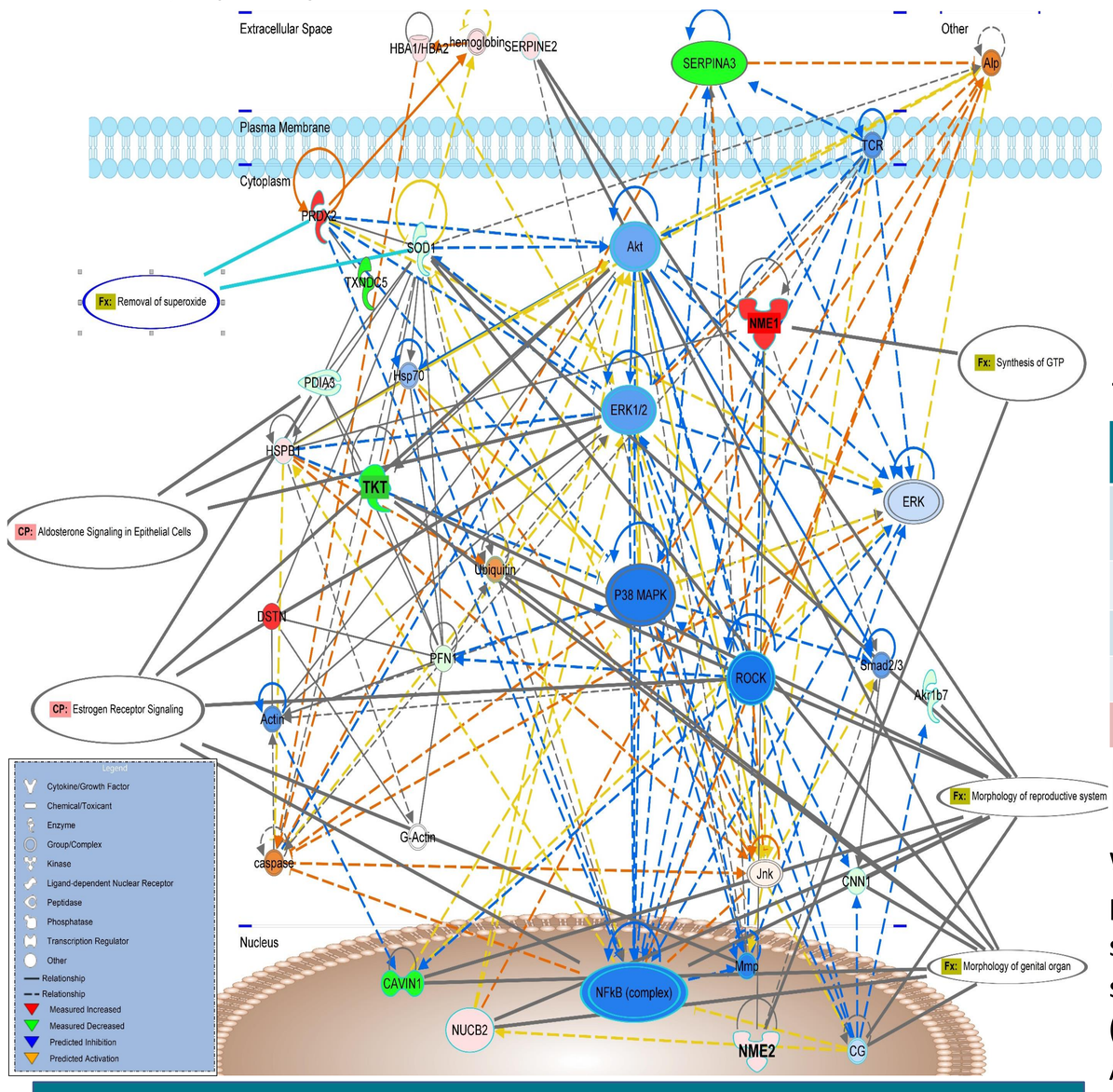


Figure 3. IPA generated network representing DNA Replication, Cellular Growth, free radical scavenging, and small molecule biochemistry. Molecular activity predictor (MAP) predicted activation of ESR2. Red and green colors represent measured increase or decrease; orange and blue represent predicted activation or inhibition, respectively.



Methods

Figure 1. Workflow of the Experiment

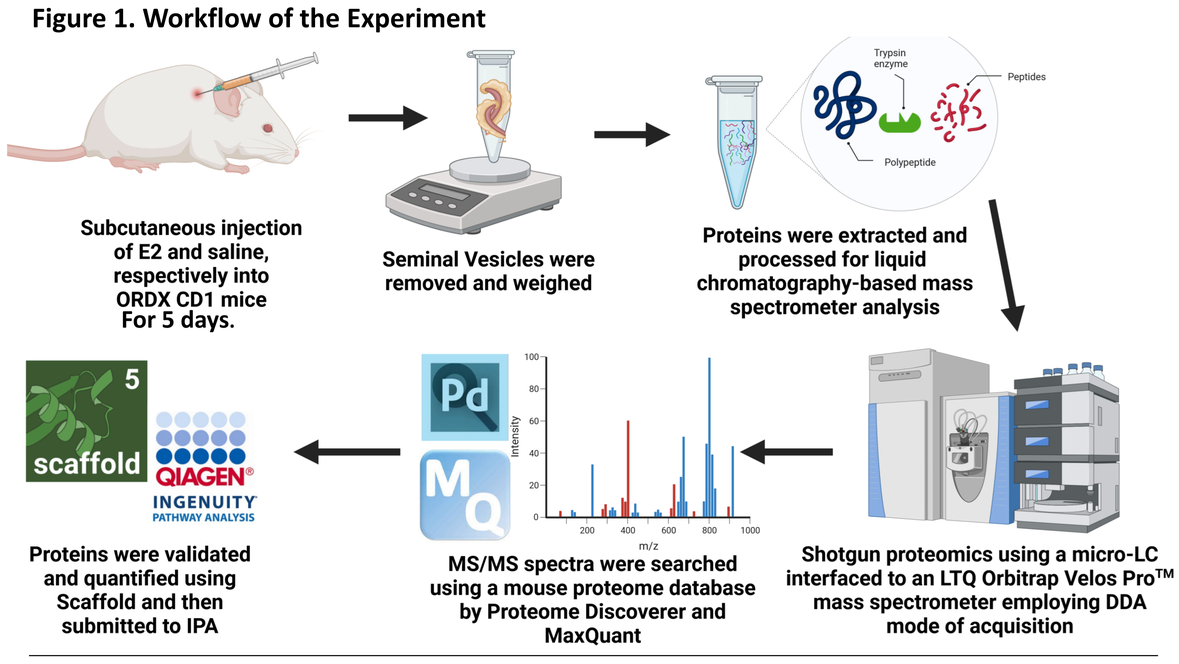


Figure 4. IPA generated network representing ESR2 activation, cell cycle, cell signaling, and small molecule biochemistry.

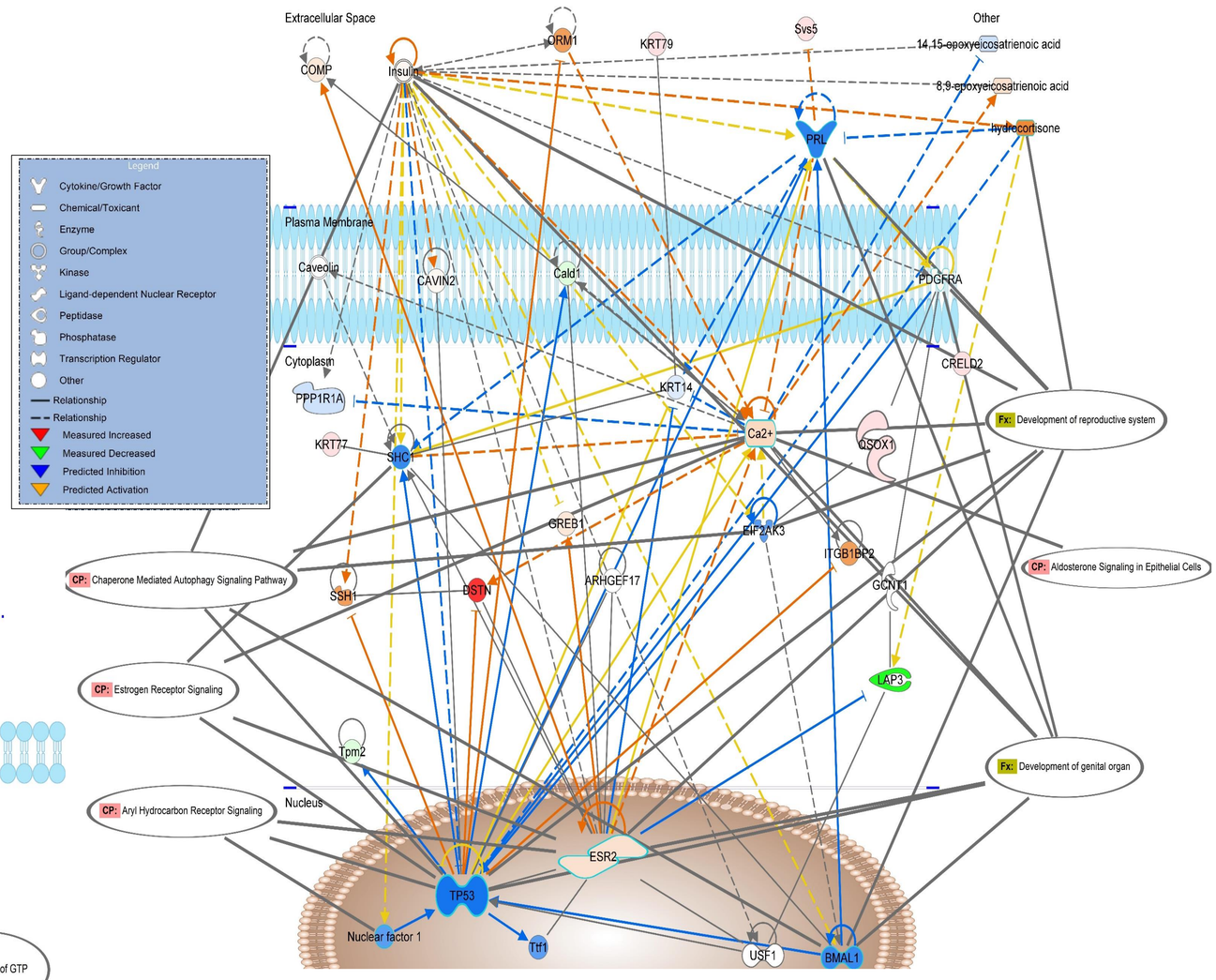


Table 1. Potential protein biomarker candidates.

Potential Protein Biomarkers	Fold Change	P-Value
Nucleoside diphosphate kinase A (NME 1)	High Expression (Not detected in controls)	3.0E-02
Nucleoside diphosphate kinase B (NME 2)	3.1	4.0E-03
Sulfhydryl oxidase 1 (QSOX1)	3.3	4.1E-02
Nesfatin-1 (NucB2)	8.2	3.0E-03
Probasin	-3.4	2.4E-02

Discussion

We reported a significant enlargement of the SV after E2 treatments **(Figure 2)**. Our proteomic analysis identified and validated **448 proteins**. Activation of estrogen signaling through ER-β (ESR2) was predicted in the MAP-enhanced network suggesting that ER signaling induced the observed change in protein expression **(Figure 3)**. Several proteins involved in cellular growth and proliferation including AKT, MAPK and NFκB, were predicted to be **inhibited** by E2 treatment **(Figure 4).** Proteins such as **NucB2**, **QSOX1, NME1 and NME2** had markedly increased expression between treatments and, with further validation, may be utilized as potential biomarkers for estrogenic signaling that affects the SV **(Table 1)**.

Acknowledgments

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