How Bipartite Network Visualizations Complement Ingenuity Pathway Analysis: A Case Study in Methylation Related to Preterm Births

Suresh K. Bhavnani PhD[1], Bryant Dang BSc[1], Maria A. Caro MSc[1], Ram Menon PhD[2]
[1]Inst. for Translational Sciences, [2]Department of Obstetrics and Gynecology, UTMB, TX

Abstract

Although bipartite networks have been effective in identifying how significant biomarkers are associated to different subsets of patients, little is known about how domain experts use such patterns to infer biological pathways. Here we present a case study to elucidate how patterns from a bipartite network visualization were used in conjunction with Ingenuity Pathway Analysis (IPA) to infer pathways related to methylation in spontaneous preterm birth. The results suggest that because the network made explicit the inverse symmetrical relationship among cases/controls and methylation sites, the visualization helped to rapidly integrate pathways related to cases and to controls into a unified pathway hypothesis for spontaneous preterm. These results elucidate the complementary role that bipartite networks can play in inferring biological pathways from databases such as IPA.

Introduction

While bipartite networks have been effective in helping comprehend complex associations among subjects and biomarkers, little is known about how such information is used by domain experts to infer biological pathways. We therefore posed the question: *What information in a bipartite network of cases/controls and methylation sites is useful in identifying biological pathways from IPA?*

Method

In a recent study[1], we generated a bipartite network of 22 preterm cases (24-34 weeks gestational age) and 28 controls (>39 weeks gestational age) and the top-10 significant DNA methylation sites from a whole-genome study of cord blood of African American subjects. As shown in Figure 1, the network and subsequent cluster analysis revealed a patient cluster on the left with all but 2 cases that were hypermethylated (represented by dark gray edges) at 7 sites, and a patient cluster on the right with mostly controls that were hypermethylated at 3 sites. This network, along with IPA-generated pathways for each subset of methylated sites was provided to an expert in preterm biology. The expert was asked to think aloud while he attempted to identify the pathways, and his verbal protocol was recorded and analyzed to identify the steps taken to arrive at a hypothesis.

Results and Conclusion

The domain expert first attempted to analyze the IPA-identified pathways for 7 hypermethylated sites in the left cluster consisting of most cases. Unfortunately, none of the pathways appeared to be meaningful in preterm. Next, he analyzed the IPA-identified pathways for the 3 hypermetylated sites in the right patient cluster consisting of mainly controls. Here he determined that 2 hypermethylated sites (cg23754392, cg25592206) on genes BMI1 and CDKN2C respectively, which could be downregulated (due to being hypermethylated) resulting in the upregulation of TP53 (a known tumor suppressor identified by IPA) leading to normal cell senescence required for the normal rupture of the placenta during labor. Because these very sites were hypomethylated (represented explicitly by the light gray edges that connected the cases to these two sites) in most of the cases, he inferred that the opposite might hold for the cases: hypomethylation of the same 2 sites would lead to expression of BMI1 and CDKN2C, leading to the suppression of TP53 which in turn would result in minimal or absent cellular senescence, requiring surgical rupture of the placenta during preterm labor. Having determined a plausible role of cellular senescence in preterm, he reexamined the genes related to the left cluster of hypermethylated sites in the cluster of most cases. This led to a focus on BCL9 and IRF8, both of which are cell cycle promoters. He therefore unified the two insights by concluding that the absence of the pathway related to cellular senescence, in combination with the presence of a pathway that promoted cell cycle might be responsible for the preterm cases. These results elucidate how bipartite networks, which can explicitly represent inverse symmetrical relationships, can aid in rapidly integrating pathways related to cases and controls into a unified pathway hypothesis.

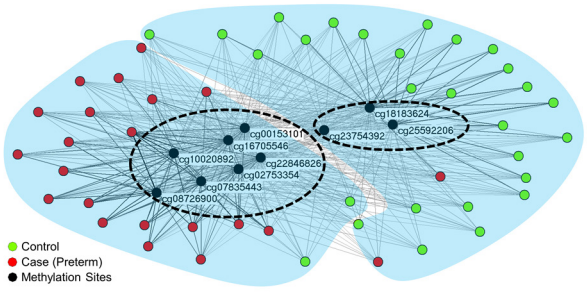

Figure 1. Bipartite network of 50 cases/controls, and top-10 significant methylation sites. Subject clusters are shaded in blue; methylation site clusters are marked with dotted ovals.

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References

1. Bhavnani et al. Methylation Differences Reveal Heterogeneity in Spontaneous Preterm Birth Pathophysiology: A Visual Analytical Approach (*in press*).