Dana-Farber Cancer Institute

Comparing Subtypes of Breast Cancer Using a Message-Passing Network

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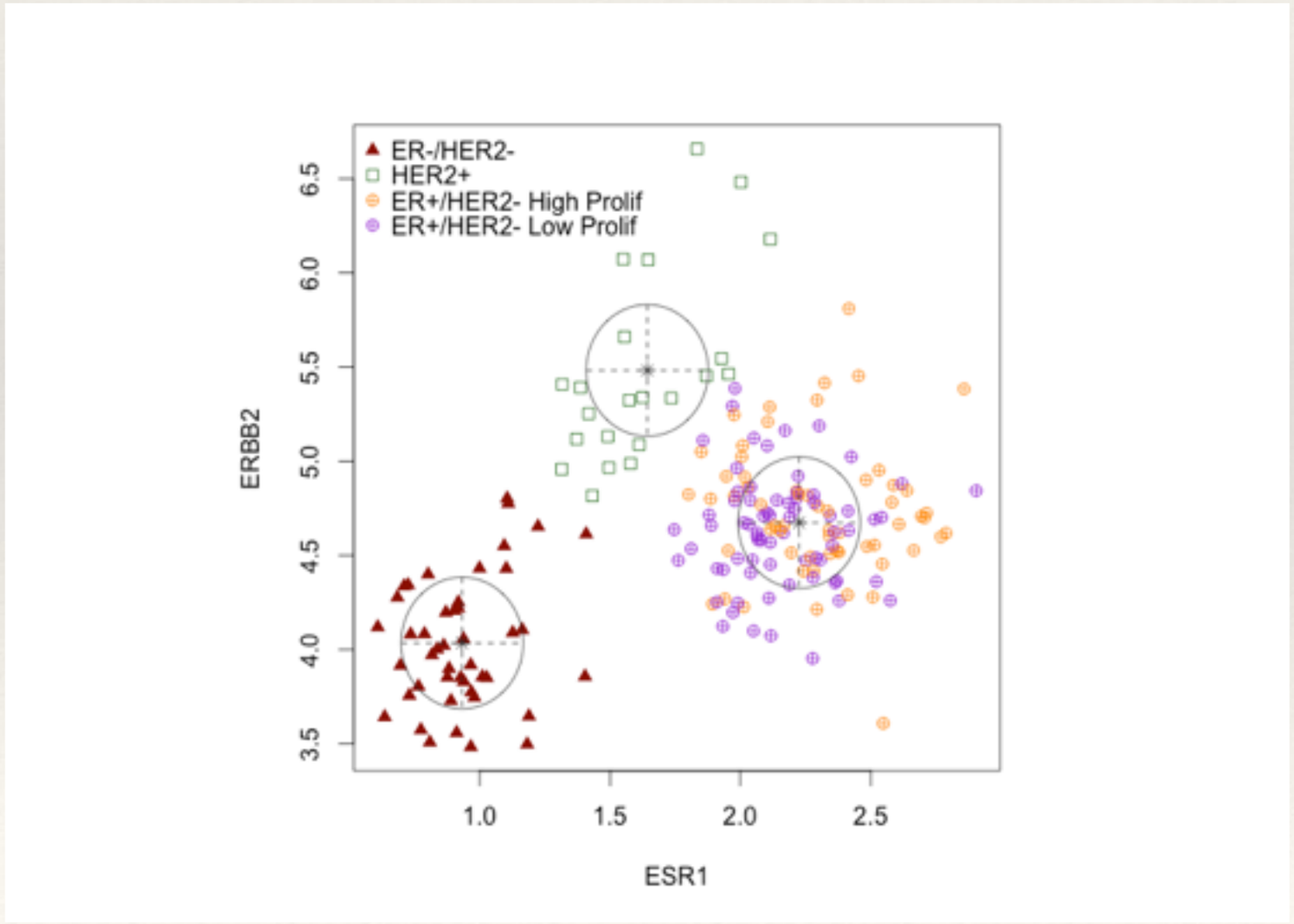
Outline

- Breast cancer and its molecular subtypes
- Passing Attributes between Networks for Data Assimilation (PANDA)
- Luminal A vs. Luminal B breast cancer
- Basal-like vs. Luminal B breast cancer Gene Sets
- Future goals

Molecular Subtypes of Breast Cancer

- Four recognized molecular subtypes of breast cancer:
  - Luminal A
  - Luminal B
  - Basal (also called Triple Negative BC)
  - HER2-positive (ERBB2)

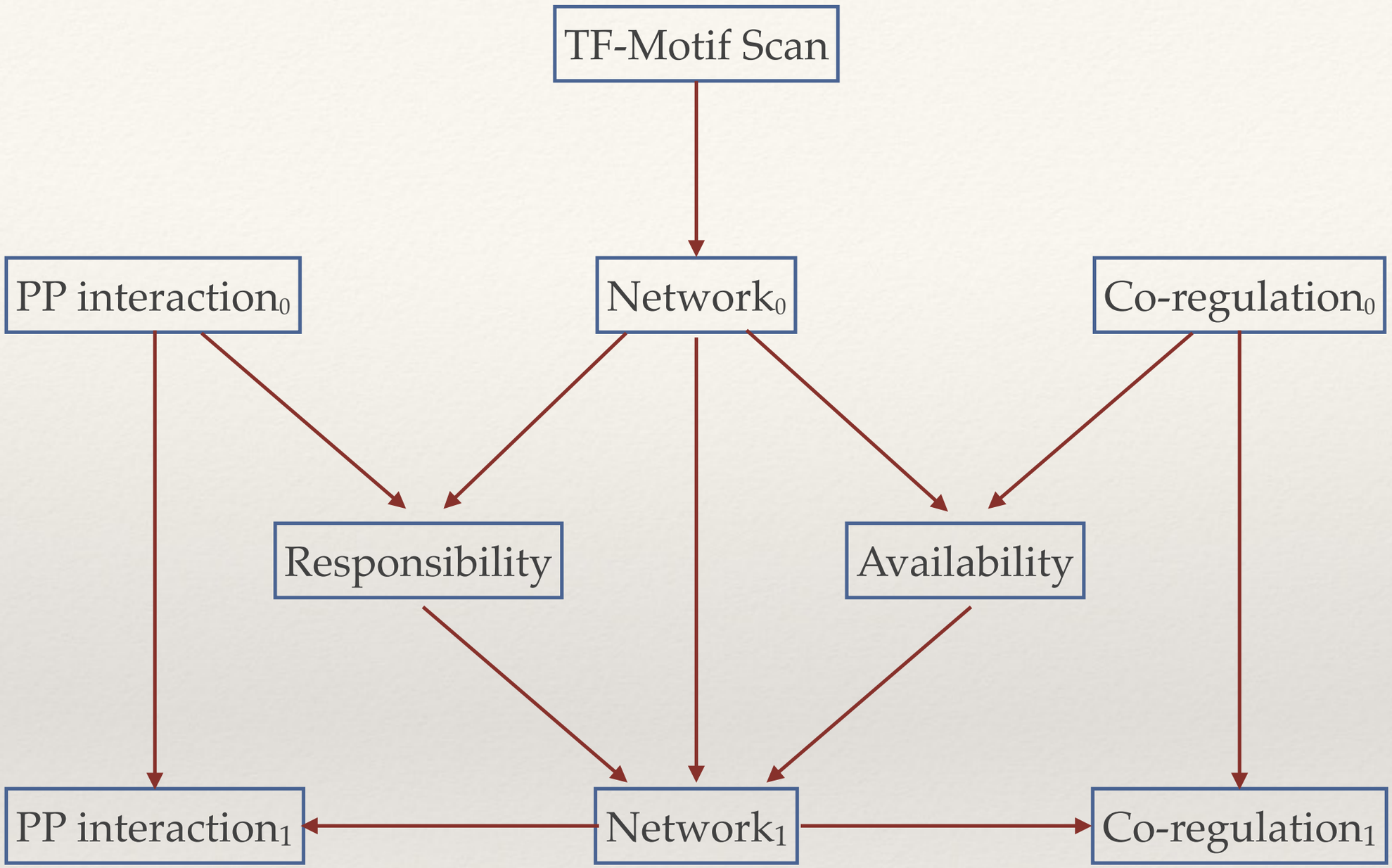
- 198 samples came from *Bioconductor* public website, data is Breast Cancer TRANSBIG
- Samples were separated according to molecular subtype using the Subtype Clustering Model[1]



71 samples were Luminal A, 60 samples were Luminal B, 45 samples were Basal-like breast cancer, and 22 samples were HER2+. Luminal A and Luminal B are distinguished by level of proliferation.

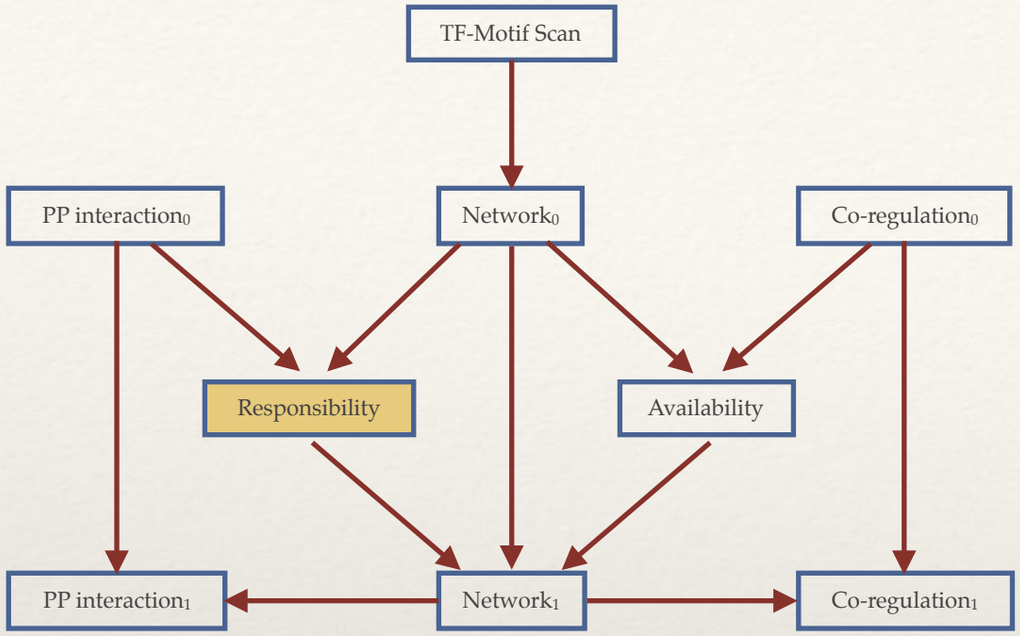
PANDA Algorithm[2]

- Main objective of PANDA is to find *agreement* between data represented by multiple networks:
  - Protein-protein interaction
  - Gene-expression (co-regulation network)
  - TF-gene interaction (regulatory network)



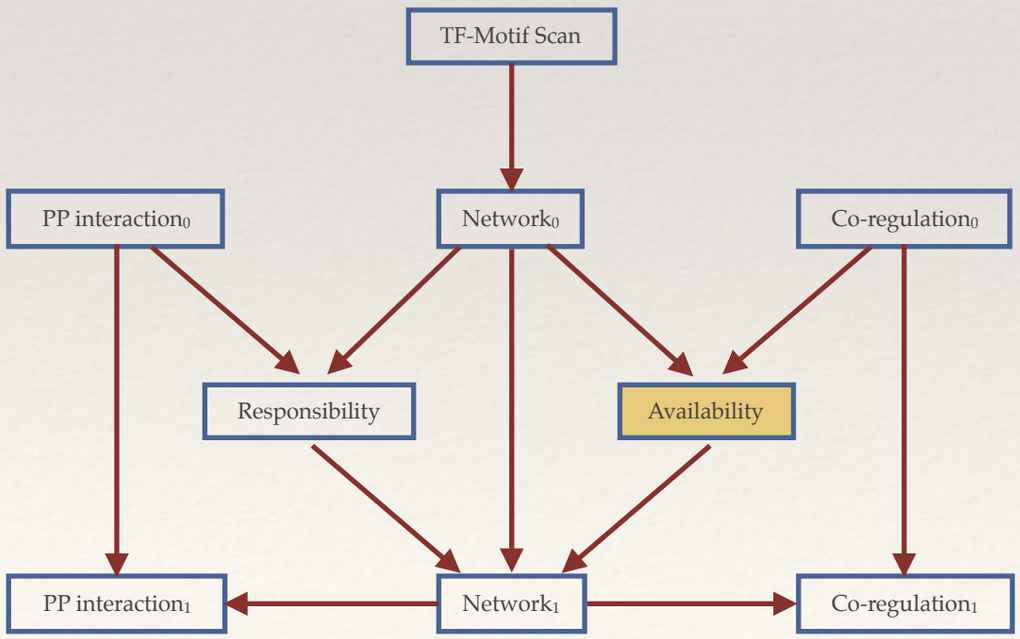
PANDA estimates the probability that an edge exists in a network and returns that estimate in terms of Z-score units

- *Responsibility(R_{ij}):* information flowing from TF *i* to gene *j*



$$R_{ij}^{(t)} = \frac{\sum_m P_{im}^{(t)} W_{mj}^{(t)}}{\sqrt{\sum_m \left(P_{im}^{(t)}\right)^2 + \sum_m W_{mj}^{(t)^2} - \left|\sum_m P_{im}^{(t)} W_{mj}^{(t)}\right|}}$$

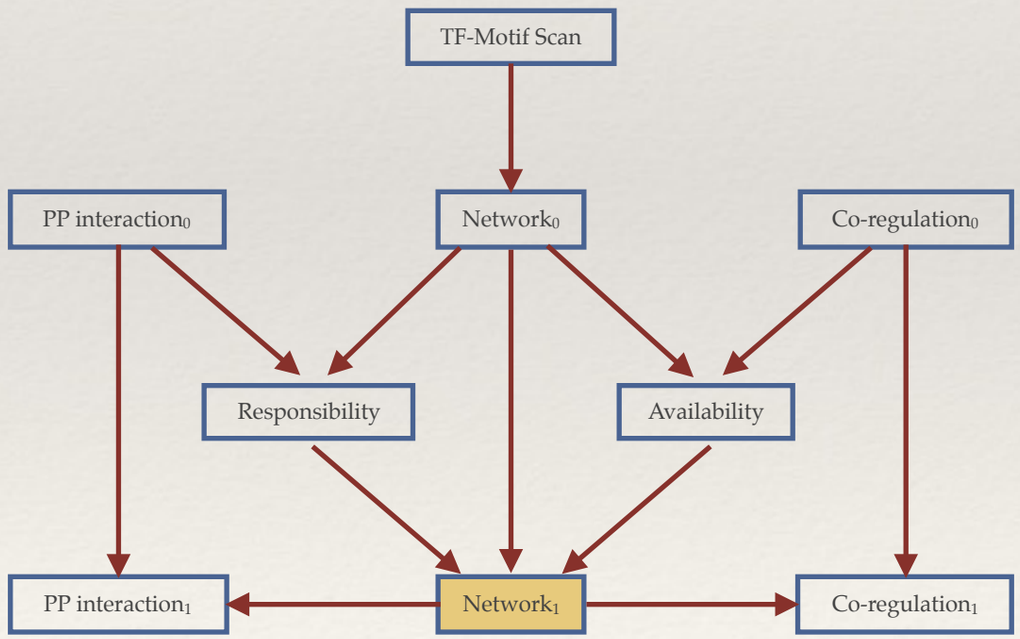
- *Availability (A_{ij}):* information flowing from gene *j* to TF *i*



$$A_{ij}^{(t)} = \frac{\sum_k W_{ik}^{(t)} C_{kj}^{(t)}}{\sqrt{\sum_k \left(W_{ik}^{(t)}\right)^2 + \sum_k \left(C_{kj}^{(t)}\right)^2 - \left|\sum_k W_{ik}^{(t)} C_{kj}^{(t)}\right|}}$$

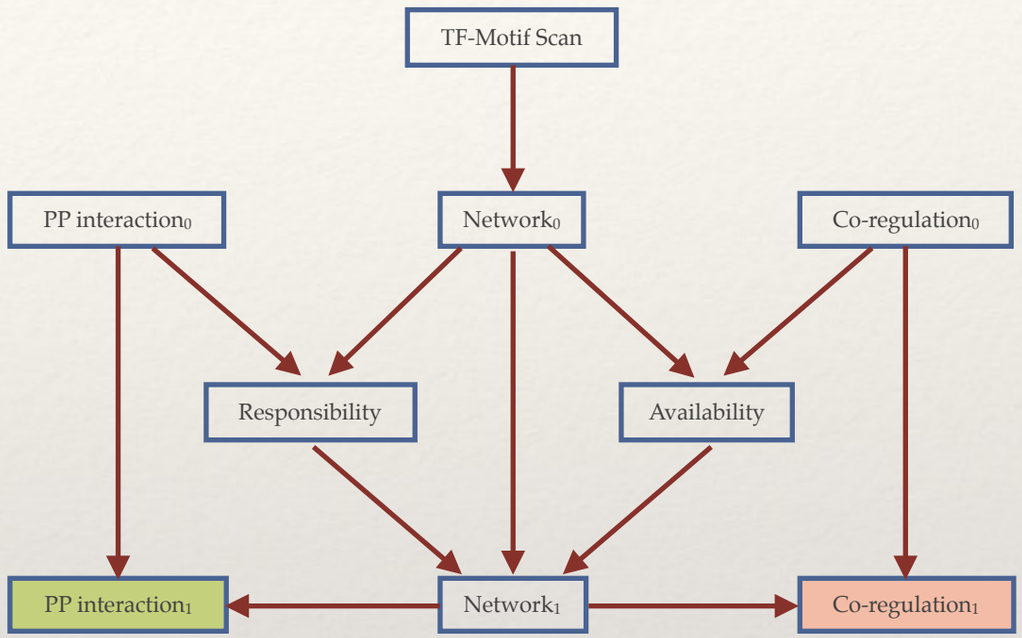
- Since regulation requires both that TF is responsible for the regulation of a certain gene and that gene to be available for regulation by that TF:

$$\tilde{W}_{ij}^{(t)} = \frac{A_{ij}^{(t)} + R_{ij}^{(t)}}{2} \qquad W_{ij}^{(t+1)} = (1-\alpha)\, W_{ij}^{(t)} + \alpha \tilde{W}_{ij}^{(t)}$$



- Cooperation between TF's *i* and *m*:

$$\tilde{P}_{im}^{(t)} = \frac{\sum_j W_{ij}^{(t)} W_{mj}^{(t)}}{\sqrt{\sum_j \left(W_{ij}^{(t)}\right)^2 + \sum_j \left(W_{mj}^{(t)}\right)^2 - \left|\sum_j W_{ij}^{(t)} W_{mj}^{(t)}\right|}}$$



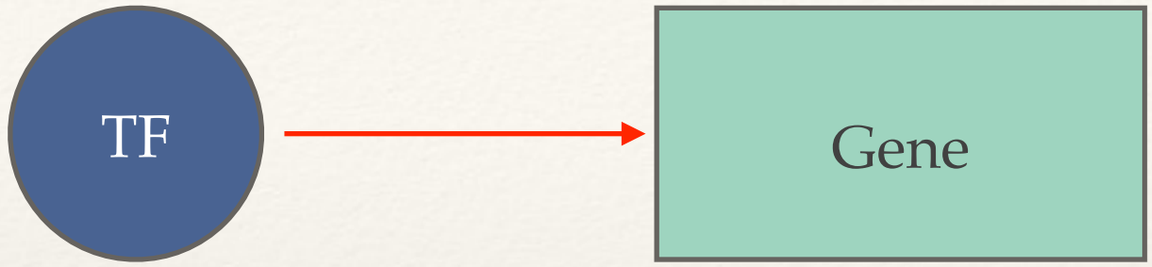
- Co-regulated genes *j* and *k*:

$$\tilde{C}_{kj}^{(t)} = \frac{\sum_i W_{ik}^{(t)} W_{ij}^{(t)}}{\sqrt{\sum_i \left(W_{ik}^{(t)}\right)^2 + \sum_i \left(W_{ij}^{(t)}\right)^2 - \left|\sum_i W_{ik}^{(t)} W_{ij}^{(t)}\right|}}$$

- Update matrices:

$$P_{im}^{(t+1)} = (1-\alpha)\, P_{im}^{(t)} + \alpha \tilde{P}_{im}^{(t)} \qquad C_{jk}^{(t+1)} = (1-\alpha)\, C_{jk}^{(t)} + \alpha \tilde{C}_{jk}^{(t)}$$

Luminal A vs Luminal B: Transcription Factors

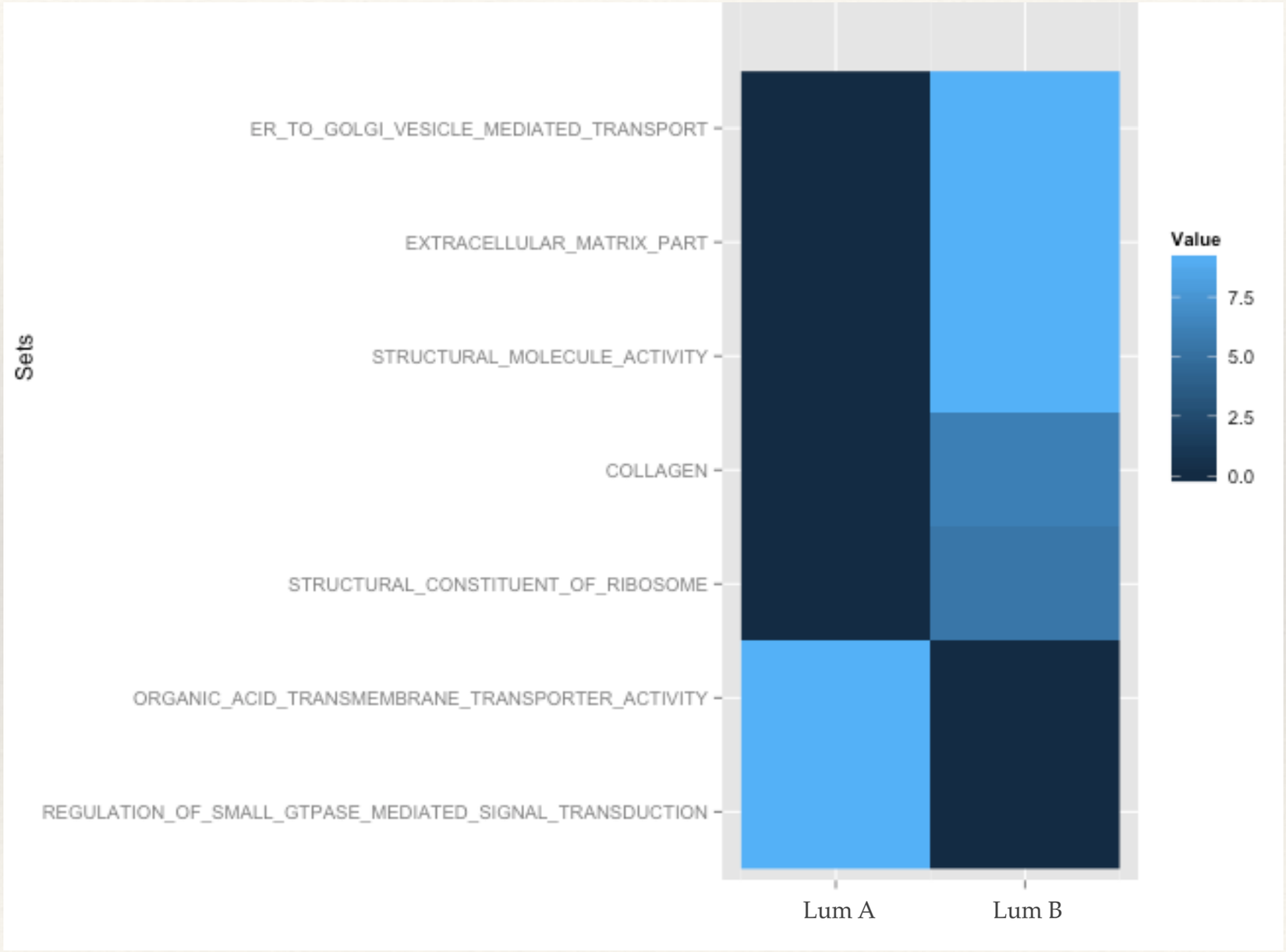


- We looked at the transcription factors with the highest differential targeting between the two networks with p-value < .001
- 25 key transcription factors (1 in Luminal A and 24 in Luminal B). At least 6 of 24 are considered proto-oncogenes or interact with one
- *CREB1* - binds to *cAMP* response element, stimulates transcription
- *ELK1* can augment the growth suppressive function of *BRCA1a/1b* proteins in breast cancer cells[3]. However, data did not show a relationship with any *BRCA* gene, but it did with *ELK4*

- *HINFP (MIZF)* - interacts with *MBD2* and plays role in DNA methylation (it has been shown that *MBD2* functions as a demethylase to activate transcription)[4]
- *JUN::FOS* - mutation in regulator increases metastasis; study suggests development of anti-c-Jun strategies in breast cancer therapy[5]

Luminal A vs Luminal B: Gene sets

Gene Set Enrichment Analysis[6]. Genes were ranked using differential regulation, then gene list was run on GSEA where genes were assigned to a gene set. Gene sets involved with extracellular structure and protein transportation are enriched in Luminal B.



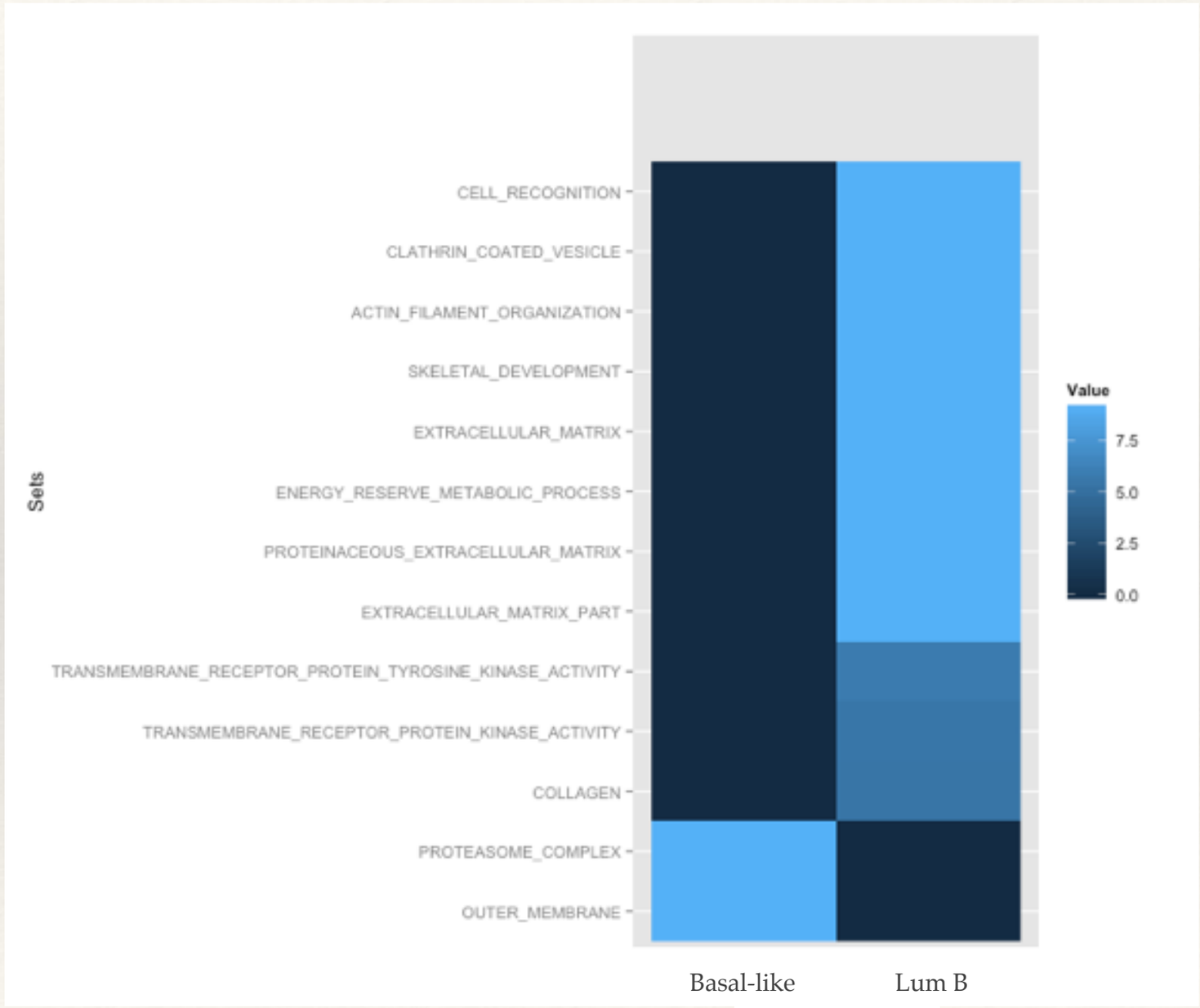
$$Value = -\log_{10}(p.value)$$

Luminal A vs Luminal B: Gene Expression

- *AURKA, BRCA2, CDK4, MYBL2* (cell cycle progression), *MKI67*, and *CCNB1*(involved in mitosis) are expressed significantly higher in Luminal B than in Luminal A
- *PTEN*, a negative regulator and tumor suppressor, is expressed significantly lower in Luminal B.
- *MKI67* index could serve as a potential proliferation marker that could successfully differentiate Luminal A from Luminal B[7]
- *CDK4* has role in proliferation, cyclin inhibitors are currently in early-phase development[8]
- *FGFR1* gene amplification in Lum B (not significant), knockdown of *FGFR1* could reverse resistance to endocrine therapy[9]

Basal vs Luminal B: Gene Sets

- Luminal B has enriched gene sets involved with extracellular activity, structure, and cell recognition. Basal-like BC has enriched gene sets involved with mitochondrial activity[10] and proteolysis (breaking down of proteins)



Future Goals

- Expand function/method of PANDA:
  - Currently bipartite network
    - Effects of genes on genes
  - Network Structure
- Differences among most aggressive subtypes

Acknowledgments

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References

1. Haibe-Kains, B., Desmedt, C., Loi, S., Culhane, A.C., Bontempi, G., Quackenbush, J., Sotitiou, C. (2011). A Three-Gene Model to Robustly Identify Breast Cancer Molecular Subtypes. *Journal of the National Cancer Institute Advance Access, Vol. 104,* 1-15. doi: 10.1093jnci/djr54
2. Glass K, Huttenhower C, Quackenbush J, Yuan G-C (2013) . Passing Messages between Biological Networks to Refine Predicted Interactions. *PLoS ONE 8(5): e64832*. doi:10.1371/journal.pone.0064832
3. Chai, Y., Chipitsyna, G., Cui, J., Liao, B., Liu, S., Aysola, K., …, Rao, V. (2001). c-Fos Oncogene Regulator ELK1 Interacts with BRCA1 splice variants BRCA1a/1b and enhances BRCA1a/1b - mediated growth suppression in Breast Cancer Cells. *Oncogene, 20,* 1357-1367.
4. Fujita, H., Fujii, R., Aratani, S., Amano, T., Fukamisu, A., Nakajima, T. (2003). Antithetic Effects of MBD2 Regulation. *Molecular and Cellular Biology,* 2645-2657. DOI: 10.1128/MCB.23.8.2645-2657.2003.
5. Zhang Y, Pu X, Shi M, Chen L, Song Y, Qian L, Yuan G, Zhang H, Yu M, Hu M, Shen B, Guo N (2007). "Critical role of c-Jun overexpression in liver metastasis of human breast cancer xenograft model". *BMC Cancer* **7**: 145. doi:10.1186/1471-2407-7-145
6. http://www.broadinstitute.org/gsea/index.jsp
7. Cheang, M., Chia, S., Voduc, D., Gao, D., Leung, S., Snider, J., …, Nielsen, T.O. (2009).Ki67 Index, HER2 Status, and Prognosis of Patients With Luminal B Breast Cancer. *JNCI J Natl Cancer Inst* 101 (10): 736-750. doi: 10.1093/jnci/djp082
8. Cyclin D1 Kinase Activity Is Required for the Self-Renewal of Mammary Stem and Progenitor Cells that Are Targets of MMTV-ErbB2 Tumorigenesis. *Cancer Cell , Volume 17 , Issue 1* , 65 -
9. Turner N, Pearson A, Sharpe R, Lambros M, Geyer F, Lopez-Garcia MA, Natrajan R, Marchio C, Iorns E, Mackay A, Gillett C, Grigoriadis A, Tutt A, Reis-Filho JS, Ashworth A: FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer**.** *Cancer Res 2010,* **70:**2085-2094
10. Kim, M., Kim, D., Jung, W., Koo, J., (2013). Expression of metabolism-related proteins in tripe-negative breast cancer. *Int J Clin Exp Pathol*, 7(1): 301–312.