

# Context-dependent utilization of serine in cancer

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Serine and glycine have diverse biological functions but the general and context-dependent utilization of these nutrients in cancer is poorly understood. Our recent work integrates genomics data and isotope tracing using computational tools to study serine utilization across multiple cancer and normal human samples.

Serine and glycine are amino acids with diverse cellular functions. Notably, serine and glycine donate carbon units and fuel the serine, glycine, one-carbon (SGOC) metabolic network. Through their metabolism across the SGOC network, these nutrients support a wide variety of downstream cellular processes such as nucleotide synthesis, methylation metabolism, sulfur metabolism, polyamine metabolism, lipid and protein synthesis, and redox balance.<sup>2</sup> In recent years, we and others have reported multiple instances where serine uptake or its *de novo* synthesis is significantly elevated in tumor cells.<sup>3-6</sup> It was shown that several human cancer types, including breast and colorectal cancers, rely on serine for proliferation and survival and that hyperactivity in the network can drive the development of cancer. Utilization of serine to increase *de novo* nucleotide synthesis rates in highly proliferative cells has been the main explanation proposed for this observation. However, serine and glycine feed into numerous other metabolic pathways. Furthermore, recent studies have shown that serine is also used for the production of NADPH and that the role of serine in the regulation of redox status could be critical to cell proliferation.<sup>7-10</sup> In parallel to these recent functional studies, unprecedented amounts of molecular-level data have been collected on primary human tumors through the efforts of The Cancer Genome Atlas (<http://cancergenome.nih.gov>).

These data allow comprehensive, systematic analysis of the population structure of human cancer and can result in unbiased assessments of molecular mechanisms when analyzed in a statistically rigorous manner. In order to better understand the functions of serine in cancer pathogenesis, we constructed a network of genes related to SGOC metabolism and studied the usage of the network across many cancer contexts.

With analysis across hundreds of human cancer samples spanning 4 major cancer types it became possible to carefully study many aspects of the network in cancer. The network was analyzed at the levels of individual genes and functional pathways. Functional pathways were defined using serine as the input and measurable products that achieve biologically distinct outcomes (Fig. 1). Beyond studying the expression of genes and pathways, the correlation structure of the network was also investigated. Positive correlations among genes generally give insights into their functional relatedness whereas negative correlations could indicate that the pair of genes might be used for different activities. Similar reasoning can also be applied to functional pathways when they are defined as the set of metabolic genes encoding enzymes that complete a series of reactions that convert serine to a distinct product.

With these metrics established, we were able to interpret gene expression in the

network. Our analysis first confirmed that nucleotide synthesis pathways were universally upregulated in all cancer types compared to corresponding normal tissues. Furthermore, we observed a strong positive association between the expression of nucleotide synthesis pathways and pathways mediating oxidation and reduction, including the synthesis of glutathione and NADPH. A negative correlation was always observed between taurine and nucleotide synthesis pathways. In addition to what appeared to be general features of the network, tissue-specific pathway expression patterns were also apparent. The majority of serine utilizing pathways showed heterogeneity in expression both between and within cancer types. This apparent heterogeneity in the network suggests the existence of subtypes of metabolism, with some tumors, for example, devoting their one-carbon units to methylation in favor of performing other network tasks.

A major caveat thus far is that this work only extends to gene expression. Metabolism, however, involves protein expression, enzyme activity, and coordinated regulation of enzymes in a pathway that results in a flux. Thus, the closest readout related to a biological phenotype in metabolism is a flux. To investigate the relationship between gene expression and flux, we developed a method to estimate fluxes using high-resolution mass spectrometry data.

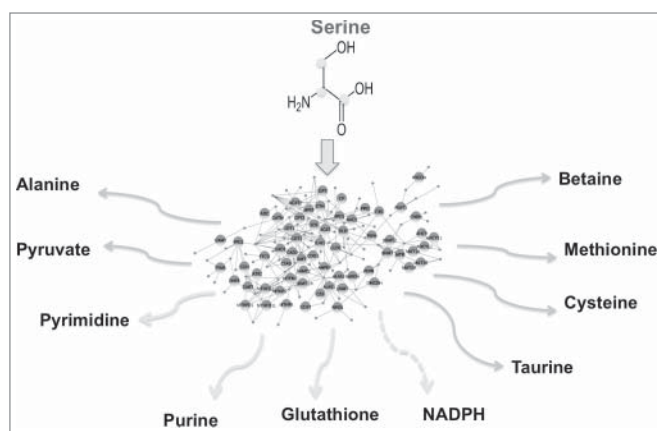
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**Figure 1.** Decomposition of the SGOC network into pathways with measurable outputs. Yellow arrows show correlated flux through the nucleotide and glutathione pathways. The yellow dashed line to NADPH indicates that we indirectly verified that NADPH production flux correlated with nucleotide and glutathione synthesis.

A panel of cell lines was cultured in stable isotopically-labeled serine and then the metabolites were extracted. The labeling patterns of metabolites corresponding to many of the outputs in the SGOC network were measured. A mathematical model was then applied to estimate fluxes from the obtained labeling patterns or mass isotopomer distributions in the SGOC network.

We found that expression of individual genes poorly predicted the metabolite labeling patterns; however, the collective expression of genes in a pathway turned out to be a better predictor of flux. Therefore, by considering the overall expression of genes in a pathway, one might be able to achieve a sense of the flux through the pathway. This finding probably has more general implications in the study of

metabolism but further analysis is needed to ascertain the extent to which gene expression can serve as a biomarker of metabolic flux. Insights from the flux study showed that serine was simultaneously used in nucleotide synthesis and glutathione synthesis pathways, confirming a positive correlation between redox and nucleotide metabolism (Fig. 1). This implies that tumor cells might need to coordinate their redox status in order to maintain high rates of nucleotide synthesis and repair.

Despite these advances, many questions remain. One aspect of serine metabolism that was not thoroughly considered is phospholipid metabolism. Furthermore, it is not clear whether the heterogeneity of pathway utilization identified in our study could be used in predicting chemotherapy response when multiple agents that target the pathway are used clinically. Nevertheless, this study provides a framework for deeper analysis of these key questions in cancer metabolism.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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