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Original Research Article

Genome scale metabolic modeling of cancer

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ABSTRACT

Cancer cells reprogram metabolism to support rapid proliferation and survival. Energy metabolism is particularly important for growth and genes encoding enzymes involved in energy metabolism are frequently altered in cancer cells. A genome scale metabolic model (GEM) is a mathematical formalization of metabolism which allows simulation and hypotheses testing of metabolic strategies. It has successfully been applied to many microorganisms and is now used to study cancer metabolism. Generic models of human metabolism have been reconstructed based on the existence of metabolic genes in the human genome. Cancer specific models of metabolism have also been generated by reducing the number of reactions in the generic model based on high throughput expression data, e.g. transcriptomics and proteomics. Targets for drugs and bio markers for diagnostics have been identified using these models. They have also been used as scaffolds for analysis of high throughput data to allow mechanistic interpretation of changes in expression. Finally, GEMs allow quantitative flux predictions using flux balance analysis (FBA). Here we critically review the requirements for successful FBA simulations of cancer cells and discuss the symmetry between the methods used for modeling of microbial and cancer metabolism. GEMs have great potential for translational research on cancer and will therefore become of increasing importance in the future.

1. Introduction

Cancer is a neoplastic disease, where cells are reprogrammed to avoid the checkpoints in control of nutrient supply, growth, aging, death and dissemination (Hanahan and Weinberg, 2011). This is often caused by genetic events, e.g. mutations and copy number alterations, affecting the cell's signaling system. Cancer cells develop the ability to rapidly evolve, by acquired genomic instability. This allows the cancer cells to adapt to new environments allowing it to metastasize, to evade the immune system and resist cancer treatments. Aberrant energy metabolism is involved in many human diseases (Sangar et al., 2012) and reprogramming energy metabolism is an emerging hallmark of cancer (Hanahan and Weinberg, 2011).

Genome scale metabolic models (GEMs) attempt to collect all metabolites and metabolic reactions catalyzed by the enzymes of the genome in a unified mathematical framework. They have been successfully applied to microorganisms to unravel genotype-phenotype relationships, e.g. the lethality of gene knockouts (Duarte et al., 2004), and a wide range of algorithms have been developed (Lewis et al., 2012). Many methods center around Flux Balance Analysis (FBA), where the metabolic state of the cell is described by the biochemical reaction rates (Fig. 1A–C). GEMs have been extended to human metabolism (Fig. 1D) and employed in the study of cancer, as extensively reviewed before (Edelman et al., 2010; Bordbar and Palsson, 2012; Jerby and Ruppin, 2012; Lewis and Abdel-Haleem, 2013; Sharma and König, 2013; Ghaffari et al., 2015a; Mardinoglu and Nielsen, 2015; Masoudi-Nejad and Asgari, 2015; Resendis-Antonio et al., 2015; Yizhak et al., 2015). These reviews were focused on the reconstruction of generic models as well as cell or disease specific models (Bordbar and Palsson, 2012), applications for patient stratification, personalized treatment and drug target identification (Lewis et al., 2010; Mardinoglu and Nielsen, 2015), the heterogeneity of cancer (Ghaffari et al., 2015a; Resendis-Antonio et al., 2015), the biology of cancer metabolism and known mutations (Sharma and König, 2013; Yizhak et al., 2015) and labeling experiments (Lewis and Abdel-Haleem, 2013). This review aims to cover these topics whilst paying special attention to the requisites for FBA modeling, and how FBA modeling of human cells differs from the modeling of microbial cells.

We begin this review by discussing alterations in metabolic enzymes affiliated with cancer. We then proceed to methods and applications of GEMs, both methods that already have been employed in cancer research and methods that have been developed for microbes

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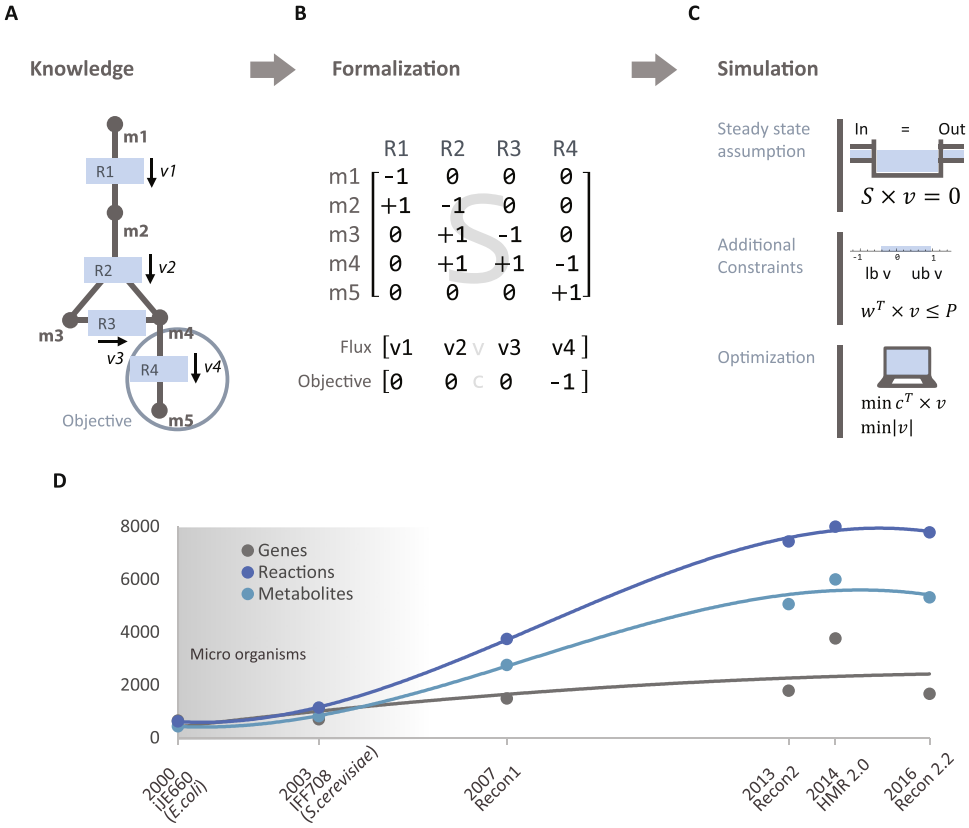

Fig. 1. Biological knowledge is formalized into a mathematical representation on which hypotheses can be tested and simulations run. (A) Assembly of biological knowledge about biochemical reactions, **R**, and rates, **v**, the participating metabolites, **m**, and biological driving forces in form of biomass equations and cellular objectives. (B). Formalization of the metabolic network as a stoichiometric matrix, **S**, where reactions are represented as columns, metabolites as rows and the stoichiometry in the reactions represented as the non-zero elements. (C) Metabolic simulations commonly use the steady state assumption, where the metabolites produced by one reaction are immediately consumed by another reaction, preventing buildup of internal metabolites. Additional constraints may also be added e.g. flux bounds on exchange reactions. Using linear programing, flux distributions can be identified that optimize the flux through the objective function. (D) The amount of genes, reactions and metabolites represented in genome scale models have increased with time and with the shift to more complex organisms. The models we have today are rather comprehensive and new content is added at a slower rate.

with translational potential. We pay special attention to quantitative flux studies using FBA and review the experimental parameters that enable accurate flux predictions. We end the review by evaluating the limitations and possibilities of GEMs in general and FBA in particular. We conclude that GEMs are useful tools to structure knowledge of cancer metabolism and for hypothesis generation, testing and validation.

2. Cancer metabolism

The increased proliferation rates in cancer cells require corresponding adaptations to the metabolic fluxes. It is debated (Ward and Thompson, 2012) whether metabolic changes are driving the cancer progression (the supply model) or if they are merely a response to increased consumption rates (the demand model). It is reasonable to assume that both mechanisms may be involved, and many metabolic genes and regulators of metabolism are implied in cancer (Fig. 2). Metabolism of cancer has been reviewed before (Vander Heiden et al., 2009; Yizhak et al., 2015; Ward and Thompson, 2012; Cairns et al., 2011). We here draw from these reviews to present some key metabolic features of cancer, falling into the two categories; rapid ATP generation, and increased biosynthesis of macromolecules.

2.1. Synthesis of ATP

Free energy in the form of ATP is fundamental to cellular growth and is required for polymerization of macromolecules as well as other growth related activities (Verduyn et al., 1991), and the growth rate of mammalian cells in culture is a function of the ATP formation rate (KILBURN et al., 1969). The synthesis of ATP takes place in glycolysis, the TCA cycle and oxidative phosphorylation. For each glucose molecule metabolized 2 ATP molecules are obtained from glycolysis, 2 from the TCA cycle, and around 21.6–26 by oxidative phosphorylation.

It may therefore seem counter intuitive that most tumors experience the Warburg effect, where oxidative phosphorylation is bypassed and glucose is fermented to lactate (Cairns et al., 2011). The cells appear to compensate for this by increasing the uptake flux, making glucose consumption, measured by positron emission tomography (PET), a reliable marker for tumor detection, present in >70% of all tumors (Cairns et al., 2011). Neither oxygen limitations, nor mitochondrial defects appear to be causing the phenotype (Cairns et al., 2011).

It has been hypothesized that the increased glucose flux mainly serves as a source for reduced carbon required for biosynthesis (Ward and Thompson, 2012; Heiden Vander et al., 2011), but both stoichiometric analysis and labeling experiments have ruled out that option (Shlomi et al., 2011; Hosios et al., 2016; Keibler et al., 2016). A similar phenomenon, dubbed overflow metabolism or aerobic fermentation, is present in many microorganisms, where glucose is converted to different fermentation products, also in the presence of oxygen. Mitochondrial capacity constraints or macromolecular crowding have been proposed drivers (Sonnleitner and Käppeli, 1986; Beg et al., 2007; Molenaar et al., 2009), and metabolic modeling suggest that this also may be the case for tumor cells (Shlomi et al., 2011; Vazquez and Oltvai, 2011). This is corroborated by the fact that also muscle cells and other metabolically active cells produce lactate as a response to increased ATP demand (Vazquez and Oltvai, 2011; Vazquez et al., 2011).