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Development of a sampling-based global sensitivity analysis workflow for multiscale computational cancer models

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Abstract: There are two challenges that researchers face when performing global sensitivity analysis (GSA) on multiscale ‘in silico’ cancer models. The first is increased computational intensity, since a multiscale cancer model generally takes longer to run than does a scale-specific model. The second problem is the lack of a best GSA method that fits all types of models, which implies that multiple methods and their sequence need to be taken into account. In this study, the authors therefore propose a sampling-based GSA workflow consisting of three phases – pre-analysis, analysis and post-analysis – by integrating Monte Carlo and resampling methods with the repeated use of analysis of variance; they then exemplify this workflow using a two-dimensional multiscale lung cancer model. By accounting for all parameter rankings produced by multiple GSA methods, a summarised ranking is created at the end of the workflow based on the weighted mean of the rankings for each input parameter. For the cancer model investigated here, this analysis reveals that extracellular signal-regulated kinase, a downstream molecule of the epidermal growth factor receptor signalling pathway, has the most important impact on regulating both the tumour volume and expansion rate in the algorithm used.

Nomenclature

Abbreviations

ANOVA	analysis of variance
EGF	epidermal growth factor
EGFR	EGF receptor
ERK	extracellular signal-regulated kinase
GSA	global sensitivity analysis
LSA	local sensitivity analysis
MAPK	mitogen activated protein kinase
MEK	mitogen activated protein kinase kinase
MLRA	multivariate linear regression analysis
PLC γ	phospholipase C γ
PKC	protein kinase C
PRCA	partial rank correlation analysis

1 Introduction

Recently, computational cancer models across different biological scales, that is, ‘multiscale’ cancer models, have garnered much attention for its potential to help move the

field of integrative cancer systems biology towards clinical implementation [1–3]. As the model parameters defining biological properties at different scales are generally not produced by a single laboratory, studying the dynamic system behaviours governed by a fixed set of parameters is inappropriate. This implies that the influence of the perturbations of these parameters on the overall system behaviour needs to be further investigated [4]. Sensitivity analysis has been widely accepted as a useful tool for this purpose, especially when it is not possible or practical to conduct numerous wet-lab experiments [5]. There are two types of sensitivity analysis methods: ‘local’ sensitivity analysis (LSA) and ‘global’ sensitivity analysis (GSA). As the LSA (one-at-a-time parameter variation method) only allows one parameter to change each time, for analysing complex biosystems, such as cancer, GSA is believed to be more appropriate for accessing a parameter’s sensitivity because it allows multiple parameters to change simultaneously.

Thus far, a number of GSA techniques have been developed, especially in the engineering field, and include response surface methodology, Monte Carlo analysis (sampling-based approach), and variance decomposition

procedures (variance-based approach). GSA methods have also been applied to systems biology models [4, 6–8], but most of them focus on the analysis of signalling pathways. To assess the context-dependent relationship between different biological scales of interest, we have previously provided an applicable GSA strategy based on the integration of Monte Carlo and resampling methods as well as the repeated use of analysis of variance (ANOVA) [9]. Read *et al.* [10] also developed a GSA method based on statistical techniques to link simulation results back into the original biology domain in order to determine the confidence of the simulation-derived predictions. However, there is no single ultimate solution that best fits all types of systems biology applications, that is, each method has its advantages and disadvantages [11]. A particular method may be favoured over another depending on the specific model being studied and the objectives of the analysis. Hence, in identifying inputs critical for certain outputs, it is better to consider multiple methods together.

In this paper, we present a sampling-based GSA ‘workflow’ that accounts for multiple GSA methods together. We chose sampling-based GSA methods because they are relatively easy to implement and to demonstrate the applicability of the workflow; the focus here is not on whether or not variance-based methods are always more superior over sampling-based ones. Specifically, in addition to the ANOVA-based method, two other sampling-based GSA methods, that is, partial rank correlation analysis (PRCA) and Sobie’s [12] multivariate linear regression analysis (MLRA; capable of prioritising parameters for non-linear computational models), are also an integral part of the workflow. After an initial parameter ranking is produced with a specific GSA method, parameters are grouped by ANOVA using statistical comparison procedures. In the end, we generate a summarised parameter sensitivity ranking sorted by the strength of influence that each input parameter exerts on model output. We exemplify the feasibility of the workflow using a 2D multiscale agent-based model previously developed for simulating non-small cell lung cancer (NSCLC) [13]. The identified critical model parameters (on the molecular level) may have the potential to serve as therapeutic targets in treating NSCLC.

2 Methods

2.1 Multiscale NSCLC model

The two-dimensional (2D) agent-based NSCLC model [13] encompasses both molecular and microscopic (i.e. multi-cellular) scales, and we only briefly introduce its key development methods here. An epidermal growth factor (EGF)-induced, EGF receptor (EGFR)-mediated signalling pathway is implemented at the molecular scale, and includes seven main components (see Fig. 1a for an illustration of the simplified pathway). At the microscopic scale, a lattice-based 2D biochemical microenvironment is constructed and populated with diffusive chemical cues including EGF, glucose and oxygen (see Fig. 1b for model setup). Each cell (or agent) in the model carries a self-maintained signalling pathway, and as a simulation run progresses, these cells constantly sense changes in environmental factors, interact with other cells and adjust their behaviour according to a set of predefined biological rules. A molecularly-driven cellular phenotypic decision algorithm (Fig. 1a) is established to determine cell

phenotypic transitions upon molecular changes: phospholipase C γ (PLC γ)-dependent migration and extracellular signal-regulated kinase (ERK)-dependent proliferation (see [13] for detail). This algorithm is derived from and supported by experimental studies [14, 15]. In the model, each lattice grid can be occupied by one cell or remain empty at a time; if a cell decides to migrate or proliferate, it will search for a neighbourhood location to move to or for its offspring to occupy. The model is able to quantify the relationship between extracellular stimuli, intracellular signalling dynamics and multi-cellular tumour growth and expansion. Thus, it can be used to investigate the cross-scale effects of ‘simultaneous’ molecular parameter variations on tumour ‘outcome’ at the microscopic scale.

2.2 GSA workflow

We propose a GSA workflow consisting of ‘three’ phases (Fig. 2): pre-analysis (for preparing the basic input sampling data set), analysis (for performing sensitivity analysis with three GSA methods and for quantifying the distribution of the sensitivity index) and post-analysis (for producing the final summarised parameter ranking). We will explain each phase using the 2D NSCLC multiscale model (described in Section 2.1) as a practical example. Note that, to investigate the effects of how molecular changes in individual cancer cells percolate throughout and across the scales of a cancer system, the model output (i.e. biological response of the tumour) no longer consists of the behaviours of output signals or signal activation patterns (as it is in most current signalling pathway studies [16]); instead, the output is the tumour’s growth and expansion rate, two phenotypic behaviours at the microscopic level driven by the implemented molecular network. Similar to

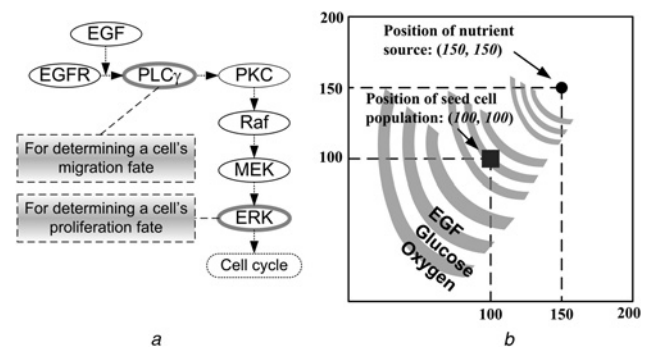


Fig. 1 Introduction of the 2D agent-based model

a Schematic representation of the signalling pathway

In short, EGFR is activated by binding to extracellular EGF, inducing receptor dimerisation and autophosphorylation

The bound receptor forms a docking site for the signalling molecule PLC γ , which then activates the Raf signal through PKC

This process initiates the ERK signalling cascade, which is involved in cellular proliferation, differentiation and survival. The rates of change of PLC γ and ERK are employed to determine cell migration and proliferation chances for the next step

b Virtual 2D microenvironment with a discrete lattice containing 200 \times 200 grid points

A single, distant blood vessel representing a ‘nutrient source’ is located at (150, 150)

The nutrient source is the most attractive location for the chemotactically-acting tumour cells (i.e. cells tend to move towards the nutrient source)

When the first cell reaches the nutrient source, a simulation run is terminated. The diameter of each cell and the unit interval of the 2D microenvironment are all 10 μ m

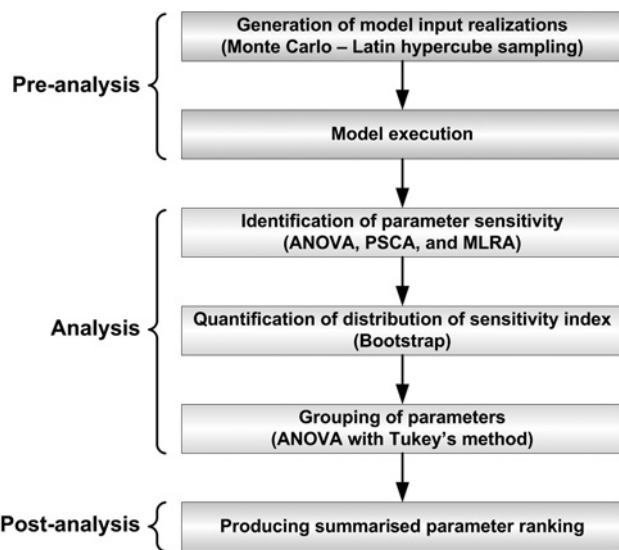


Fig. 2 Workflow of the global cross-scale sensitivity analysis, which is composed of three phases: pre-analysis, analysis and post-analysis

previous studies [17–19], we will use the number of elapsed time steps as a measure for ‘tumour expansion rate,’ and the final number of live cells for ‘tumour volume.’

2.2.1 Pre-analysis: Continuous input parameters are first partitioned into mutually exclusive ranges of values, and each individual range is termed a parameter level. In our case, we only consider the initial concentrations of pathway components as input parameters to demonstrate the applicability of the GSA workflow. When the number of parameters or parameter levels is large, exploring the entire parameter space is computationally impractical. For example, suppose we have K parameters and for each parameter we have N levels, for a total of N^K combinations. The number of simulations grows exponentially as K or N increases. The other fact rendering exploring the entire space impractical is that this/any 2D multiscale cancer model takes a relatively long time to finish, because each cell has to undergo a series of pathway analysis throughout the course of the simulation. Hence, we use a ‘random sampling’ of input parameters to render the large number of variation combinations computationally manageable. Specifically, as implemented in [9], we use the Latin hypercube sampling (LHS) method to generate 2000 random sets of parameter values, and thus 2000 sets of simulation results will be generated correspondingly. For simplicity, we call each set of parameter values along with the corresponding two tumour output values an ‘observation’. Thus, at the end of the pre-analysis phase, we have 2000 observations, and we refer to the 2000 observations as the ‘original’ sample.

2.2.2 Analysis: The sensitivity indices for the three sampling-based GSA methods are as follows: the F value for ANOVA calculated by the F -test [9], the magnitude of the partial correlation coefficient for PRCA, and the magnitude of the regression coefficient for MLRA. For all analyses, the bigger the value of the sensitivity index is, the larger the influence that the (molecular) parameter has in determining the (microscopic) system output. We note here that, based on the original sample obtained at the pre-analysis phase, each of the three GSA methods can

already yield their own parameter rankings. However, there is still the possibility that the sample data is biased (regardless of how sophisticated the LHS method is), which would make the resultant ranking incorrect. Thus, we further quantify the ‘sampling distribution’ of the sensitivity index for each GSA. As implemented in [9], to understand such distributions (of F value, partial correlation coefficient and regression coefficient, respectively), we use bootstrap resampling [20], which repeatedly samples the original sample with replacement and forms a new sample that is the same size as the original sample. The most attractive feature of this approach is that we do not have to run the multiscale cancer model again, thereby saving a great deal of time. In practice, we again generate 1000 bootstrap samples (including the original sample) and then apply each GSA to each bootstrap sample to calculate the sensitivity index values. As a result, with respect to each model output (tumour volume or expansion rate), for each GSA, there will be 1000 sensitivity index values (each corresponding to a bootstrap sample) generated for each input parameter. We then draw probability distributions of sensitivity indices from these results.

Next, to discriminate between two closely ranked input parameters, we use ANOVA with Tukey’s method (also known as Tukey’s studentised range test) [21]. Tukey’s method is a single-step multiple pairwise comparison procedure which, in conjunction with ANOVA, can determine which parameter means across the groups are significantly different from the others. In brief, suppose we have N treatment groups: ANOVA examines the difference across the N group means as a whole, and Tukey’s method looks for statistically significant differences between each pairs of the groups. In our case, each input parameter is regarded as a treatment group, containing 1000 sensitivity index data. We note that other statistical methods for performing multiple comparison tests, such as Duncan’s test, Scheffe’s procedure and the Waller-Duncan k -ratio t test can be used as a substitute for Tukey’s method; however, detailed discussion of this topic is beyond the scope of this paper, and interested readers should refer to [22]. If the difference between the means of two originally closely ranked input parameters is statistically significant (unless otherwise noted, all statistical comparisons are conducted at the 5% confidence interval), they will be assigned to different groups; if not, they will remain in the same group. Parameters from the same group are assumed to have similar effects on the model output, and thus will be reassigned the same rank. In practice, for all three GSA methods, a rank of 1 is assigned to the input with the highest sensitivity index in a parameter ranking, and the largest value of rank is assigned to the input of least importance (i.e. lowest sensitivity index).

2.2.3 Post-analysis: Since there is no one-stop GSA method for all types of systems applications, we propose an approach to synthetically take into account the parameter rankings produced by all of the GSA methods. This approach calculates the weighted mean of the rankings for each input parameter, according to the following equation

$$S_i = \sum_{j=1}^N k_j r_{i,j} \quad (i = 1, 2, \dots, M) \quad \text{and} \quad \sum_{j=1}^N k_j = 1$$

where i refers to the i th of M parameters, j refers to the j th of N GSA methods; $r_{i,j}$ represents a specific rank for the i th

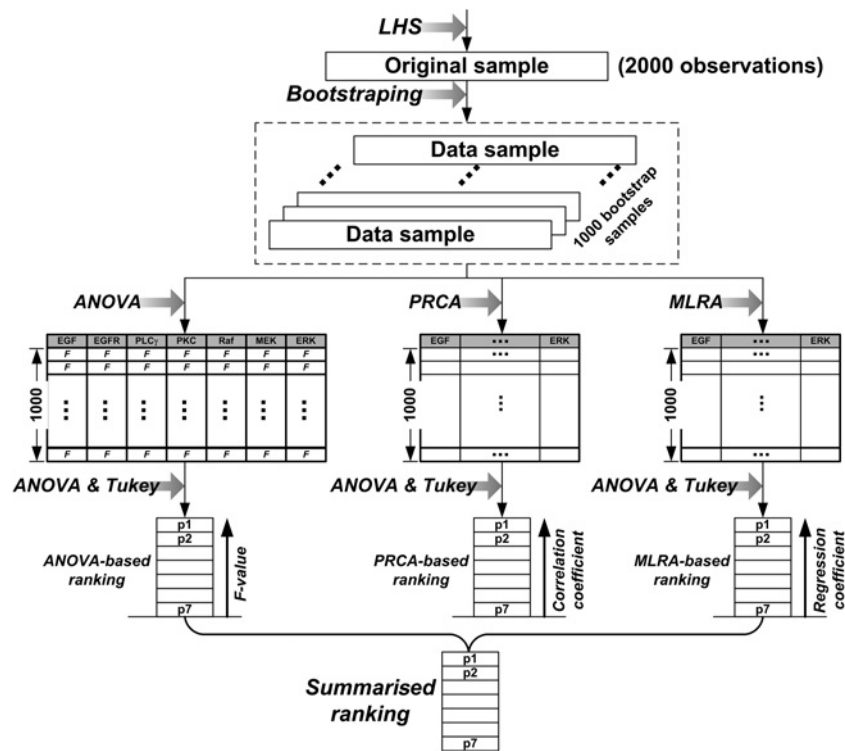


Fig. 3 Process for producing the final summarized parameter ranking

All three GSA methods (ANOVA, PRCA and MLRA) generate their own individual parameter rankings based on the same 1000 bootstrap samples. Note that all GSA methods use ANOVA and Tukey’s method to perform parameter grouping. The summarised ranking is obtained using Eq. (1)

parameter with respect to the j th GSA method; k_j represents the weight for the j th GSA method; and S_i is the final score for the i th parameter. The smaller the S_i , the more important is the given parameter. The weights are normalised to sum up to 1. Fig. 3 illustrates the process of producing the summarised (or integrated) parameter ranking. In practice, GSA methods can be set with different weights (i.e. the coefficient k), depending on the researchers’ experience or prior knowledge. For example, for a specific model, methods that have been proven to be more suitable than others may have higher weights, while others have lower weights. However, for simplicity and because we have no a priori knowledge regarding which GSA method is more powerful than others for this particular multiscale cancer model, we set $k_j = 1/N$ ($j = 1, 2, \dots, N$), meaning that all GSA methods are equally important.

3 Results

The agent-based model was implemented in C/C++. In each simulation, a total of 49 seed cells arranged in a 7×7 square were initially positioned in the 2D lattice. The first 2000 sets of parameter combinations (i.e. the original sample) were created with Matlab 2008 (Mathworks, Inc.). All statistical analysis programs for running ANOVA, Tukey’s method and bootstrap resampling were developed with SAS/STAT 9.3 (SAS Institute). Each GSA method took approximately 3 min to obtain the ranking results on a Dell workstation (Pentium-4 1.7 GHz, 2.0 GB RAM). Input parameters are the initial concentrations of the seven EGFR pathway components (Fig. 1a). Table 1 summarises the input parameter variation ranges and corresponding levels.

3.1 Individual parameter rankings

For all three GSA methods (ANOVA, PRCA and MLRA), conducted using the 1000 bootstrap samples, the individual ranking results are shown in Fig. 4. All GSA ranking results find ERK to have the most significant impact on both tumour volume and expansion rate, a result which further emphasises the potential therapeutic value of ERK in suppressing overall tumour growth. In tumour volume evaluation, MEK remains among the top three parameters in all rankings, but it is far less important than ERK in influencing the tumour volume outcome because there is a big difference in each corresponding sensitivity index value between MEK and ERK. In tumour expansion rate evaluation, other than ERK, both PLC γ and EGFR are determined to be critical by all of the GSA methods. As expected, for each model output, the different GSA methods each produce different ranking results.

Table 1 Parameter variation ranges and corresponding levels of input parameters

Input	Standard value, nM	Variation range	Number of levels
EGF	2.65	0–10.0-fold	10
EGFR	80	0–2.0-fold	10
PLC γ	10	0–2.0-fold	10
PKC	10	0–2.0-fold	10
Raf	100	0–3.0-fold	12
MEK	120	0–4.0-fold	10
ERK	100	0–10.0-fold	20

Variation ranges were set large enough to cover the entire possible parameter space. The variation range for each parameter was partitioned into levels by means of evenly spaced intervals. Standard values are taken from the literature [23, 24].

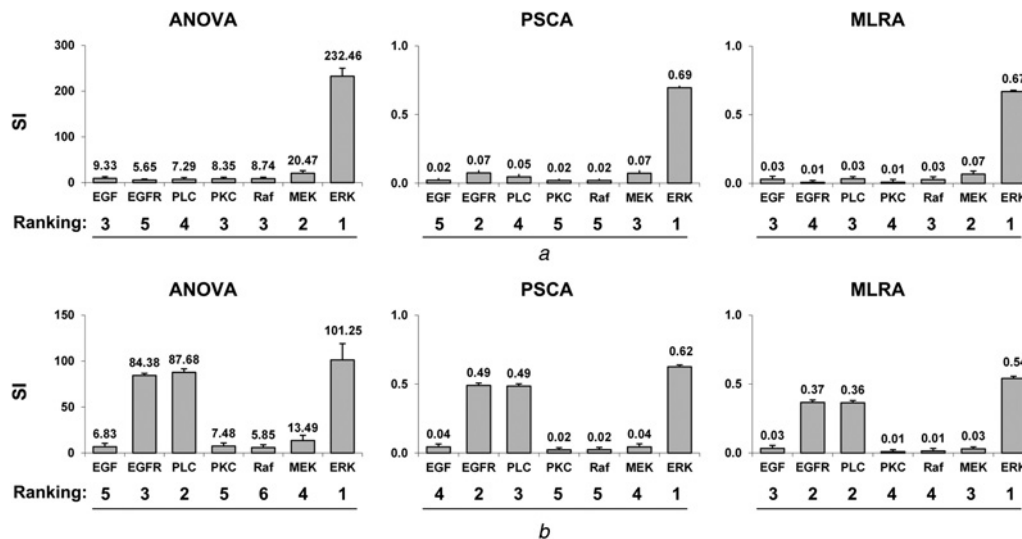


Fig. 4 Individual parameter ranking results from 1000 bootstrap simulations for ANOVA, PRCA and MLRA, with respect to
 a Final number of live cells – tumour volume
 b Number of simulation steps – tumour expansion rate
 SI stands for sensitivity index
 Columns, mean; bars, SD

3.2 Summarised ranking

For the three equally-weighted GSA methods, the value of each weight, k_j ($j=1, 2, 3$) for Eq. (1) is $1/3$. The summarised parameter rankings with respect to tumour volume and expansion rate are shown in Fig. 5. From this figure, one can easily identify whether a component is critical, as well as what the component’s position relative to other molecules in the pathway is. In tumour volume evaluation (Fig. 5a), ERK is the most critical parameter, followed by MEK. This result is most similar to the ranking obtained by using ANOVA and MLRA (Fig. 4a). That ERK is the most important parameter affecting tumour volume is not surprising, since ERK decides a cell’s proliferation fate in our phenotypic decision algorithm of the 2D NSCLC multiscale model [13]. However, in tumour expansion rate evaluation (Fig. 5b), ERK remains the most critical parameter, followed by PLC γ and EGFR. This is somewhat surprising, since we assumed that PLC γ would have the most significant impact on tumour expansion because it is the determinant of cell migration fate [13]. The identified important parameters by the final summarised ranking with respect to tumour expansion rate are in agreement with all of the three individual parameter rankings (Fig. 4b).

4 Discussion and future work

Each sensitivity analysis method (whether GSA or LSA type) has its own key assumptions, limitations and demands regarding the time and effort needed for application and interpretation [11]. With that in mind, we have presented a sampling-based GSA workflow into which a number of helper techniques, such as LHS sampling, bootstrap resampling and ANOVA with Tukey’s method for parameter grouping are introduced, and have applied the workflow to a previously developed multiscale NSCLC model [13]. Overall, the workflow provides solutions to (i) how to render the large number of parameter variation combinations computationally manageable, (ii) how to

effectively quantify the sampling distribution of the sensitivity index for each GSA to address the computational intensity issue and finally (iii) how to discriminate between

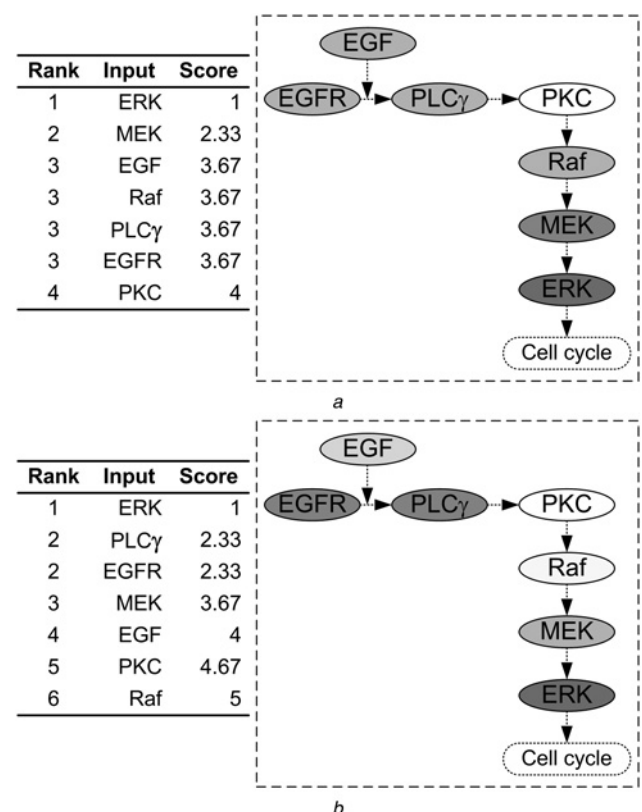


Fig. 5 Final summarised parameter ranking according to Eq. (1) with respect to
 a Tumour volume
 b Tumour expansion rate
 The ranking results (table) are shown on the left of each panel, while the right (pathway figure) identifies the potential of each component for serving as a therapeutic target
 A molecule with a higher ranking is associated with a deeper background colour

two closely ranked input parameters. Parameter ranking results indicate that, for the model used here, ERK is the most critical parameter at the molecular scale chiefly regulating the two tumour growth indices, that is, tumour volume and expansion rate, on the multi-cellular level. Cautiously extrapolated, this finding therefore supports therapeutic efforts that seek to target ERK to control tumour expansion in NSCLC. Furthermore, by extending the model to incorporate drug–cell interactions [25–29], the GSA can also be used to help develop optimal drug treatment strategies for individual patients.

The workflow introduced here is ‘flexible’ in that some methods can be substituted with others at the investigators’ choice. For example, at the pre-analysis phase, in order to introduce the uncertainty of the parameters into the model, we use LHS to randomly select parameter values from their respective probability distributions. In fact, there are many other approaches to process random sampling (see [30]), and this research topic has been extensively studied in the statistics and engineering fields. We choose to use a uniform probability distribution, that is, equal probability of selection, for all of the input parameters because the distributions of the parameters are unknown to us. If any of the parameter distributions is known a priori, this knowledge (or literature data) should be applied to the model to improve the accuracy of the parameter sampling. However, the probability distributions of parameter values for real biological systems are usually unknown, and it is thus reasonable to use a uniform distribution as the default [31]. Also, as mentioned earlier, we can employ techniques other than Tukey’s method along with ANOVA to quantify the distribution of the sensitivity index, and other types of GSA methods [11, 32] can be integrated into the workflow as well.

As noted before, it is expected that different GSA methods produce different parameter rankings with respect to either tumour growth index (i.e. tumour volume or expansion rate). This prediction is precisely what we find in our analysis, and it highlights the importance of the adjusted, summarised parameter ranking method, using Eq. (1), which integrates the individual parameter rankings. This way, a model parameter (pathway component) is identified to be critical only when all or most of the GSAs agree. In both tumour volume and expansion rate evaluation, PKC is assigned the lowest ranking by most of the GSAs, and thus is deemed to be a less important parameter. However, this conflicts with our previous LSA study’s results [17], where the model is observed to be sensitive to variations in PKC. Since the LSA method only varies a single parameter at a time while keeping all others fixed, we believe it only accesses the baseline of the effect of perturbations in each individual parameter. By incorporating multiple methods, the proposed GSA analysis procedure inherently indicates to the researcher how strong the produced ranking is – in particular, how certain the evaluation of a molecule’s importance is. A consistently high ranking indicates that a molecule is likely a good therapeutic target, since the multiple confirming analyses add to the robustness of the results. Although it may be difficult to choose the ‘best’ or ‘surest’ method of analysis per se, this ‘across-GSA methods analysis’ should increase confidence in the result, which is essential once treatment strategy choices are deduced from it.

We focus on computing the ranking of the parameters, not on understanding what level of difference would make a parameter more critical than others. In practice, this is the

researcher’s responsibility to determine this level, which also depends on the model being investigated. Although the ANOVA with Tukey’s method can divide the parameters into different groups from a statistical point of view, the result should be used only as a reference to the assessment of difference between groups.

As described, our multiscale model spans two biological scales: molecular signalling and multicellular scales; a molecularly-driven cell phenotype decision algorithm was established to link the two scales. In this study, we focused our parameter analysis on a subset of model parameters, that is, on concentrations of pathway components, simply to demonstrate the applicability of the GSA workflow. However, other scale-specific parameters (e.g. association and dissociation kinetic rates on the molecular scale, oxygen and glucose concentration profiles on the multicellular scales etc.) and the threshold parameters (specifically, for ERK and PLC γ) for linking molecular changes to cellular phenotypic determination can be varied simultaneously together with the pathway parameters to obtain a more complete ranking of pathway ‘signatures.’ Although this will incur overload computational cost, it is particularly important for a multiscale model to be useful in rationally designing multi-target or multi-component therapies. This topic has not been fully addressed yet by the multiscale modelling community.

In summary, we have presented a GSA workflow accounting for multiple GSA methods together to identify critical parameters at the molecular level that have significant impact on tumour volume and expansion rate on the microscopic level. Applying the workflow to a previously developed multiscale lung cancer model, ERK is found to be the most important molecule in regulating ‘both’ tumour evaluation indices, thus indicating its potential to serve as a therapeutic target in NSCLC. In the future, kinetic rate constants will also be considered as molecular parameters, and their cross-scale effects will be examined together with the signalling pathway component concentrations. Currently, we only use the GSA workflow to perform sensitivity analysis when a simulation task is finished, but it is reasonable to hypothesise that parameter rankings are changing over the course of the simulation. Hence, we plan to perform GSA at regularly spaced time intervals, producing a map of time-dependent ‘dynamic’ parameter rankings, which may provide additional and useful information to molecular-targeted cancer research.

5 Acknowledgment

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