SHORT COMMUNICATION

Initial assessment of a model relating intratumoral genetic heterogeneity to radiological morphology

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ABSTRACT. Tumour heterogeneity has major implications for tumour development and response to therapy. Tumour heterogeneity results from mutations in the genes responsible for mismatch repair or maintenance of chromosomal stability. Cells with different genetic properties may grow at different rates and exhibit different resistance to therapeutic interventions. To date, there exists no approach to non-invasively assess tumour heterogeneity. Here we present a biologically inspired model of tumour growth, which relates intratumoral genetic heterogeneity to gross morphology visible on radiological images. The model represents the development of a tumour as a set of expanding spheres, each sphere representing a distinct clonal centre, with the sprouting of new spheres corresponding to new clonal centres. Each clonal centre may possess different characteristics relating to genetic composition, growth rate and response to treatment. We present a clinical example for which the model accurately tracks tumour growth and shows the correspondence to genetic variation (as determined by array comparative genomic hybridisation). One clinical implication of our work is that the assessment of heterogeneous tumours using Response Evaluation Criteria In Solid Tumours (RECIST) or volume measurements may not accurately reflect tumour growth, stability or the response to treatment. We believe that this is the first model linking the macro-scale appearance of tumours to their genetic composition. We anticipate that our model will provide a more informative way to assess the response of heterogeneous tumours to treatment, which is of increasing importance with the development of novel targeted anti-cancer treatments.

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The heterogeneous composition of tumour masses has serious implications for tumour development and their response to therapy [1]. Tumour heterogeneity can result from the existence of multiple neoplastic subpopulations, each representing a distinct genotype. Clonal heterogeneity is a natural result of tumour development, with mutations in the genes responsible for mismatch repair or maintenance of chromosomal stability often occurring early in tumorigenesis [2]. Such clonal heterogeneity may manifest as differences in individual genes, DNA ploidy, histological morphology and even gross morphology [3]. It should be noted that tumour heterogeneity can also arise from variations in the tumour microenvironment [4]. Such secular heterogeneity (e.g. variations in vascularisation patterns) can also influence the response to therapy.

The relationship between clonal heterogeneity and gross tumour morphology has been investigated by Oriyama et al [5]. By studying histologically subdivided tumour regions within resected hepatocellular carcinoma specimens, they demonstrated clear differences in DNA ploidy patterns between the different regions. However, the relationship between the spatial arrangement of different foci and tumorigenesis, along with its clinical significance, does not appear to have been explored. Segal et al [6] found that 28 CT imaging traits (e.g. enhancement pattern, presence and density of necrosis, texture heterogeneity) represent 78% of expressed genes in liver cancer. However, their approach only deals with global gene expression, rather than local variations in DNA content, and so does not capture local tumour heterogeneity. Similar work has been conducted by Kuo et al [7]. Petersen et al [8], using flow cytometry, determined that colorectal carcinomas were composed of cells with different genetic content, but did not attempt to make the connection to radiological imaging and the importance of areas with similar cell types. The extensive intratumoral heterogeneity in glial tumours

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has been investigated by Misra et al [9], who used a DNA fingerprinting technique to study genomic alterations across consecutive 2 mm segments of tumour. However, extrapolating these results to relate heterogeneity to gross tumour morphology is precluded by the small size of tumours studied and the absence of any imaging data.

We have developed a biologically inspired model that enables *in vivo* exploration of tumour heterogeneity using radiological imaging methods [10]. We present our initial investigations relating morphology (from CT or MRI) to the gross genetic patterns (from array comparative genomic hybridisation (aCGH)) and its implications for assessing the response to treatment.

Model

Most current biomathematical approaches to modelling tumour growth use complex reaction–diffusion equations, requiring elaborate boundary conditions and manual tuning of the often numerous parameters in order to fit, rather than model, the anisotropic growth and physiological processes observed [11].

We have observed that, in an approximately uniform environment (*e.g.* the liver parenchyma), early stage tumours generally follow a spherical growth pattern, presumably owing to the mechanical forces from cell division. However, later stage tumours, the morphological appearance of which is often described as lobular, seem to consist of multiple connected (or overlapping) spheroids. The macroscopic continuity between individual spheres (also seen on histological examination) suggests that these lesions develop through the expansion and sprouting of spherical clonal subpopulations, forming a natural hierarchy. Accordingly, we contend that tumour development can be modelled either by the continued expansion of the original spheroid or by the sprouting of additional spheroids representing new clonal subpopulations, each with a different genetic profile. Equivalently, tumour shrinkage (*e.g.* in response to therapy) may be modelled as the reduction or disappearance of one or more spheroids at variable rates. In a uniform environment, anisotropic tumour growth is therefore a result of intratumour heterogeneity, with different clonal subtypes growing at different rates.

Figure 1. Three axial MR images (T_1 weighted three-dimensional fast gradient echo, voxel dimensions $1.8 \times 1.8 \times 5.6$ mm) taken over a 1 year period showing the development of a metastatic liver deposit. The lesion grows by expansion and sprouting of clonal centres.

To apply this model to a radiological image volume, a set of spherical objects must be positioned according to a series of steps. These steps may be performed manually or automatically using an algorithm as described in [10]. In brief, the steps are as follows.

1. Position initial spheroid randomly within tumour volume.
2. Increase size of spheroid until edge meets tumour boundary.
3. Move spheroid away from edge.
4. Repeat steps 2 and 3 until spheroid size cannot be increased further (*e.g.* edge meets tumour boundary on opposing sides).

Figure 2. (a) Pre-operative axial CT, (b) potential spherical decomposition and (c) corresponding three-dimensional model.

5. If substantial region of tumour volume not covered by spheroid remain, position additional spheroid in this region.

6. Repeat steps 2 to 5 until entire tumour volume covered by spheroids.

Figure 1 illustrates the model with three consecutive scans following the development over a period of 12 months of a metastatic Dukes B colorectal carcinoma lesion in the liver. Before treatment, the lesion appears uniform, corresponding to a single sphere. After 9 months

of chemotherapy, the second scan shows a lobular lesion, which can be modelled by the sprouting of five new spheres (clonal centres) from the original spherical clonal centre. As the new clonal centres arose during chemotherapy, they will probably consist of cell populations resistant to this treatment regime. The patient received no treatment in the following three months, and the corresponding lesion growth can be modelled by the dominant expansion of three (red outline in Figure 1) of the five spheres from the second scan.

Figure 3. Array comparative hybridisation results (blue) and corresponding segMNT lines (red) as a function of chromosome number.

Assessment

Figure 2 shows a representative axial slice from the pre-operative CT of a hypointense metastatic endometrial liver deposit (approximately $5 \times 5 \times 8$ cm). The lesion consists of multiple distinct spheroids (Figure 2b). A full three-dimensional reconstruction is shown in Figure 2c. The primary cancer was removed nine years earlier.

After resection of the right liver lobe, eight tissue samples of approximately $4 \times 4 \times 4$ mm in size were collected from five distinct regions of the tumour, each corresponding to different spheroids. For each sample, tissue was taken from the outer border of a spheroid to avoid potentially necrotic tissue. Two tissue samples were taken from each of four spheroids (turquoise, magenta, yellow and blue in Figure 2c) to assess the intra-spheroid variability. The samples were stored in liquid nitrogen prior to analysis. DNA was prepared using proteinase K digestion, phenol–chloroform extraction and ethanol precipitation [12] and sent to NimbleGen (Reykjavik, Iceland) where it was analysed using aCGH. We used a whole genome human array (2006-07-27 HG18 WG CGH) with 385 000 probes and a median probe spacing of 6270 base pairs [13]. The labelled DNA was compared with a standard female reference sample (Promega #G1521).

Figure 3 provides a coarse overview of the aCGH raw data (blue) and corresponding segmentation determined with segMNT (red) [14] for seven samples as a function of chromosome position. The eighth sample was discarded because of the resulting poor signal (the corresponding haematoxylin and eosin (H&E) stain indicated larger amounts of necrosis than for other samples). The results are most clearly seen with the segmented data and are summarised in Table 1. The segmentation algorithm shows amplification of large regions of chromosomes (1q), or entire chromosomes (7 and 10), for all of the samples. Specifically, the two samples from the turquoise spheroid are unique in their amplification of chromosome 2, while showing no amplification of chromosomes 8, 14 or 20. Similarly, the two samples from the magenta spheroid are unique in their amplification of chromosome 19. The samples from the yellow and blue spheroids differ from the other samples in their amplification of chromosome 14 but not 19, but can be distinguished from one another by the degree of amplification of region 1q.

Although our analysis currently does not allow us to assess inter- and intra-spheroid genetic variation on the basis of individual genes (owing to the quantities of tissue available), Figure 3 and Table 1 show clear differences in chromosomal amplification patterns between different spheroids, whereas amplification patterns are conserved within spheroids. These initial results, although only from a single case, lend support to our proposed model.

Assessing response to treatment

There is an increasing drive to use volumetric measurements to track treatment response as opposed to the traditional two-dimensional (2D) metrics (e.g. RECIST (Response Evaluation Criteria in Solid Tumours) [15]). However, our model implies that changes in

traditional 2D measurements or absolute tumour volume may both be misleading. The heterogeneous nature of tumours often results in a differential response to therapy, with some subpopulations responding well whereas others demonstrate resistance, continue to grow, or give rise to new resistant subpopulations [1, 9]. In such cases, the decrease in total volume or RECIST criteria may give reassurance to the clinician that the tumour is responding well to therapy, whereas in fact the disease is progressing in some areas (Figure 4). Our model could identify such a differential response *in vivo* by evaluating the change in size of each spherical subregion of the tumour, alerting the clinician to subregions that have grown in size, which may indicate the need to modify the treatment plan, possibly through the delivery of additional therapeutic agents.

Subdividing a tumour mass into discrete regions, each with potentially varied growth rates and chemosensitivity, enables a more detailed tracking of tumour development and response to therapy. In addition, specific predictions could be made with respect to the spatial expansion of the tumour. For example, in surgery, a prediction of the anisotropic expansion of a tumour may influence the determination of appropriate resection margins.

Future work

To enable a more detailed and thorough analysis, we are continuing to identify and recruit additional patients. We are also undertaking pre-clinical studies by inducing metastatic liver lesions in animal models to assess the ability of our model to track the development of liver lesions, with and without therapeutic intervention. Finally, we are analysing how information from functional imaging (positron emission tomography, dynamic contrast-enhanced MRI, blood oxygen level dependent imaging (BOLD)) can be used, in conjunction with the morphological appearance, to assess tumour heterogeneity more accurately *in vivo*. This is particularly important with the growing number of molecular targeted therapies that produce a metabolic or functional change in tumours before any anatomical effect. We have also observed a multispherical tumour composition in other tissues (e.g.

Figure 4. The spherical decomposition of lesions allows the assessment of differential response. In this example, an overall decrease in RECIST (Response Evaluation Criteria in Solid Tumours) or volume measurement may reassure the clinician that the tumour is stable or responding to therapy; however, the spherical decomposition indicates that, although two of the spheres have shrunk (green arrows), the third has expanded (red arrows) and may represent a resistant tumour subpopulation.

Table 1. Chromosomal amplification patterns for each of the tissue samples

	Chr 1q	Chr 2	Chr 7	Chr 8	Chr 10	Chr 14	Chr 19	Chr 20
Yellow	✓		✓	✓	✓	✓		✓
Turquoise 1	✓✓	✓	✓		✓			
Turquoise 2	✓✓	✓	✓		✓			
Magenta 1	✓		✓	✓	✓	✓	✓	✓
Magenta 2	✓		✓	✓	✓	✓		✓
Blue 1	✓✓		✓	✓	✓	✓		✓
Blue 2	✓✓		✓	✓	✓	✓		✓

Ticks indicate amplification, with double ticks indicating a higher degree of amplification. Chr, chromosome.

breast and brain), and hope to investigate these further. Although our work to date has focused on genetic heterogeneity, we plan to extend the model to incorporate the role of the microenvironment.

Conclusions

Our model, relating gross morphological appearance to intratumoral heterogeneity, indicates that global measures (e.g. RECIST) are insufficient to monitor treatment response. Instead, tumour-specific local measures, which account for a heterogeneous response to therapy, should be used. Our model captures lesion development and response to therapy through shrinkage, expansion or sprouting of spheroids, each representing a genetically distinct subpopulation. We have illustrated our theory with a lesion observed in consecutive MRI studies during and after the course of chemotherapy. An aCGH analysis of cancerous liver tissue demonstrated that chromosomal abnormalities (amplifications) within a spheroid are consistent, whereas substantial variation occurs between distinct spheroids. To our knowledge, this is the first time that a model linking anatomical image analysis to histological/genetic tumour heterogeneity has been proposed and assessed using clinical data. Our model enables the meaningful correlation of changes in underlying biology, morphology and therapeutic intervention, giving a more complete and personalised picture of an individual tumour, thus enhancing treatment planning and monitoring.

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