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Singularity: The Achilles' heel of cancer?

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SUMMARY

It is predicted that the total number of mutations present at the first appearance of a fully malignant clone, including passengers, is so large that every individual patient's cancer is unique from the outset. The initiating (malignant-clone-defining) mutation set (McDMS) defines the cancer, permits absolute identification of cancer cells including all sub-clones, and thus suggests a mode of attack. Directly or otherwise, a useful proportion of the McDMS will give rise to gene products that can be detected and bound by external physical agents in a specific manner. Using such agents cooperatively, as a team, offers prospects for better diagnosis and treatment, especially if they are harnessed together in one molecule so that they can all bind to their targets at the same time without strain, because that will yield enhanced selectivity and strength of binding.

Introduction

Though it has been accepted for decades that cancers are due to mutations affecting cellular control systems few people anticipated that, in addition to those driver mutations, mature tumors would also contain huge numbers of passenger mutations, non-oncogenic and of low apparent relevance to cancer phenotype or progression [1,2]. This fact, however, allows us to put flesh on a hypothesis which previously was skeletal at best; to whit, that every cancer is unique and thereby both identifiable and vulnerable. The foundations and consequences of the hypothesis are here arranged as propositions and corollaries, followed by analysis of the assumptions and calculations upon which they rest.

At the present moment it seems likely that the first catalogue of a malignant-clone-defining mutation set (McDMS) will be obtained by way of whole-genome sequencing. More eclectic approaches will be required to put such knowledge to use in treatment of patients.

Propositions and corollaries

[1] It is possible in principle to assemble a catalogue of all the mutations present at the first emergence of a fully malignant clone, the malignant-clone-defining mutation set. Corollary: A pre-malignant clone existed, containing one less driver mutation than the McDMS. Adding the last one gave rise to full malignancy.

[2] The McDMS includes passenger mutations as well as drivers, and the total number of mutations making up the McDMS is large.

[3] The McDMS is unique. From the outset the cells of a cancer can be distinguished from the host's normal tissues, and no two naturally-occurring cancers are identical in this respect. Corollaries: The cancer is delimited by the McDMS, not the genotype or phenotype of the mature tumor nor of any precursor clone. All cells of the cancer and all its sub-clones can in principle be identified absolutely by the McDMS, whatever other means of classification are imposed describing the tissue of origin, causation, stage of development and degree of malignancy.

[4] By recognizing the McDMS we identify all cells of the cancerous clone and thus distinguish them from normal tissue or other tumors or metaplastic tissues. A sufficient proportion of the McDMS will suffice. Corollary: This constitutes an increased level of discriminatory power, since we depend not on one but several markers present simultaneously, which can be thought of as independent witnesses.

[5] Directly or indirectly, a useful proportion of the McDMS will yield gene products that can be bound by external physical agents in a specific manner. Corollary: This allows us to control, remove or destroy cells of the malignant clones with improved discriminatory power, since in principle we can arrange for cooperative action of several agents, yet still attack only McDMS-dependent targets. This is a step beyond identification.

[6] If such physical agents are linked together in one molecule so that all are able to bind simultaneously without strain, improved discrimination is matched by enhanced chemical selectivity and strength of binding, which is a step beyond cooperation.

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Assumptions and caveats

Assumptions

The first group are taken to be justified without further discussion: others are more problematic and may be subject to specific caveats which if fulfilled would invalidate some propositions.

Cancers are clonal so that at every stage during the development of a tumor all successor clones bear all the mutations that were present at the origin of each clone. This is true of the McDMS as of any other mutation set. Several key somatic mutations are required to induce a fully malignant phenotype: six is believed to be commonly the minimum. These are the driver mutations from which follow functional changes that lead to both the malignant phenotype and further progressive development of the cancer including sub-clones. Mutation is random. Non-driver ('passenger') mutations also occur during the progression of a cancer to the extent that mature cancers contain thousands of somatic mutations. These are distributed at random over the genome or, if clustered, not in such a manner as to invalidate the argument.

Although it is probable that most passenger mutations are completely silent, a proportion of them will cause cellular alterations which are detectable even if lacking any functions that contribute to the malignant phenotype. It is not essential for propositions 5 and 6 that the mutations are manifested by structurally abnormal proteins at the cell surface. More subtle changes might suffice, including over-expression, under-expression or variations in the balance of products from alternative splicing of messenger RNA.

Caveats

It is conceivable that passenger mutations begin to accumulate rapidly only after establishment of the malignant clone. If so the McDMS might contain fewer passenger mutations than appear from the argument below.

Some cancers depend upon inherited mutations. In these and a few other rare types of tumor a lesser number of mutations may suffice for establishment of the malignant clone. In such cases the estimates for number of mutations in the McDMS may prove exaggerated.

It is not known what proportion of passenger mutations yield cellular alterations that are detectable by means other than whole-genome sequencing. The assumption below is less than 1 in 10 of somatic mutations which lie within the coding sequences of genes, less than 1 in 100 of mutations overall.

The progress of a cancer includes the occurrence of additional mutations after the formation of the initial set and it is possible that a member of the McDMS might be lost in such an event, yielding a sub-clone that did not contain the entire McDMS. The expectation is that this would be rare. (Conversely, sub-clones containing additional mutations are presumed to be universal and to dominate in the mature cancer. Thus a cancer is characterized by the McDMS and not by the set detected in any given sample.)

It is not certain that all mature cancers contain such huge numbers of mutations as has been reported for some types [1].

Argument

Propositions 1 and 2 assert that the minimal or originating mutation set (McDMS) will be found to contain a substantial number of passenger mutations in addition to the active oncogenes and this requires justification. Any attempt at calculation on the basis of mutation rates per generation breaks down at once because of uncertainties and difficulties of interpretation, so a conservative alternative principle is followed here.

A mature cancer contains a very large number of somatic mutations, nearly all of them passengers [1,2], but the observations so far do not allow a distinction as to whether these are within one principal malignant clone or distributed over many, and the same may be said of any driver mutations which are present in the mature cancer over and above the McDMS [1,3]. Such heterogeneity of the tumor appears at first sight to make all calculations impossible. However, the impasse is resolved if we assume that the ratio of passenger to driver mutations remains about the same throughout, including the pre-malignant phase, which must be the case if mutation occurs randomly.

There are in a mature cancer, commonly, about 20 driver mutations, 1000 mutated genes and in all over 10,000 somatic mutations [1–3].

If there are 6 driver mutations in the originating malignant clone, then we can predict 300 mutated genes and in all 3000 mutations going to make up the McDMS.

Concerning proposition 3, it will suffice to argue in support using much lower numbers of mutations than those just deduced, more nearly consonant with the number of mutations that will be detectable by means other than whole-genome sequencing. Take it that the passenger mutations of immediate interest must be within genes, easily detectable, and distributed more or less at random over a genome of 20,000 genes. Suppose that there are 10 such mutations in the McDMS (out of three hundred mutated genes). The number of ways of distributing 10 mutated genes over the entire genome is 20,000 to the power 10, more than 10^{43} – a number unimaginably large, in excess of ten million billion billion billion billion – and this is ignoring the consequences of one thousand or so alternative possible mutation sites within each affected gene.

It follows that every cancer must be unique from the outset, beginning with the McDMS.

Permutation would give a much larger number still if conducted in terms of the entire predicted number of mutations in the McDMS. This allows considerable latitude in the matter of estimating the likelihood of detecting mutations and identifying a cancer on the basis of its McDMS. Evidently only a very small proportion of the mutations actually present are required to be detectable by whatever means are employed for the purpose.

General discussion

Properly, the founder of any clone is simply the cell from which its successors are descended (each daughter cell that survives is the founder of its own clone), but is commonly thought of as the first to acquire a differentiating feature of whatever kind that persists through all succeeding generations and therefore permits recognition of the clone. In discussion of cancer development, the founder cell of the malignant clone may be equated with that in which the first aberrant mutation occurred; that is, long preceding overt malignancy. It is only to avoid confusion that the rather cumbersome phrase McDMS is used in this paper to refer to the set of mutations characterizing the first indisputably malignant cell or clone, many generations down from such a 'founder'.

It will at first be very difficult to delineate an initiating malignant clone and there will be scope for debate in any individual case about which mutations should be accepted as constituents of the McDMS. Practical difficulty and scholastic refinements can be ignored for our immediate purpose; the only thing needed is agreement that the McDMS must exist. That conclusion seems inescapable if it is true that cancer originates within a clone that was already genetically abnormal but not yet fully malignant.

The way to identify a McDMS at DNA level will be to determine the mutations present in as many separate samples as possible

from an individual cancer; very small samples, preferably each of a single cell and as varied as possible as to location and phenotype. Then the McDMS is equal to or less than the set of somatic mutations common to all samples – the lowest common denominator. Whole-genome sequencing will be valuable in this program, but may always require samples that contain many cells so that the results will continue to be blurred by the varied contributions of subclones. A method that reflects the situation in a single cell [2] will be preferred even if a full sequence is not obtained.

The literature is confusing and inconsistent on the questions of how many mutations are required for a cancer and how many passenger mutations there are. A contributory element to the dispute is failing to distinguish between initiation and the state that finally becomes observable in a patient – what is called here a mature cancer. There is general agreement from epidemiology and experiment that several driver mutations are required before a cancer begins, varying from case to case, perhaps sometimes as few as three but more often about six [1,3]. Recent observations and theory combine to indicate that the number of driver mutations in a mature cancer is greater than this [1–3] and for the order-of-magnitude calculations above we have taken 20 driver mutations (and in addition to those, 10,000 passengers). There is no real conflict between these various figures. Everything we know about cancer points to progression (more mutations and more aggression as time goes by) and clonality (cancer cells differ between themselves), predicting both that the number of mutations in each individual cell of the cancer should increase as generations succeed each other and that measurements upon a mixture of clones should yield a number of mutations greater than for an individual cell.

The McDMS is a theoretical construct which may have real existence. For everyday treatment and diagnosis a para-genetic approach will required in addition, with concentration on things easier to get at and influence than chromosomal DNA.

Thinking has for years been directed at detecting a set of abnormal cell-surface proteins characteristic of the individual cancer of an individual patient, a plan which imposes the daunting pre-condition that there should already exist a suite of antibodies or similar agents capable of binding to a sufficient range of intrinsically rare or even unique mutant epitopes. It was a relief, therefore, to understand the possibility of an approach through whole-genome sequencing [1]. Probably, the first identification of a McDMS will be by that means. Using such information for treatment is more difficult and perhaps what really happens will be a combination, with genomic information to supplement what is obtained from proteomics and detection conducted directly upon the circulating tumor cell [4] (which is intrinsically a single-cell procedure though limited to clones capable of entering the circulation). In all probability a sub-set of the McDMS will suffice to devise a treatment for an individual patient: probably also there will be commonality between patients such as to simplify the logistic problems. The definition and nomenclature of the McDMS may be shifted a little to take account of the methods actually used.

Another point of difficulty was that, even if one could identify a good set of markers in samples from a mature cancer, they might covertly be distributed over several sub-clones and thus knowledge of the whole set would not suffice for elimination of all the malignant cells. The problem is removed, in principle, by the concept of the McDMS which by definition is common to all cells of the tumor.

Proposition 4 introduces the idea of bringing several independent sources of information to bear. Such combinatory discrimination bears formal resemblance to the pattern recognition already used in diagnosis either intuitively or prescriptively and will therefore appear to pathologists as not wholly new. Much the same might be said of proposition 5 since synergism in chemotherapy is well known to be advantageous in the cases where it can be applied. In general, however, it does not seem that the variability between individual cancers has been fully taken into account in thinking about how to approach the problems. Cases are classified according to schemes that force them into artificial categories. It is thought strange that in general cancers do not display a set of oncogenic mutations characteristic of their tissue of origin or mode of causation. The propositions advanced here make it seem remarkable that they do so to even the slightest degree and indeed lead to a probable conclusion that many such mutations, perhaps especially those to *Ras* and *p53*, are close to being epiphenomena, expressing the fact that a mature and aggressive cancer now exists, saying little about how that came about and less still about how to treat it.

A natural antibody is homopolyvalent which increases its binding affinity and selectivity for the natural target. If ligands for distinct epitopes are combined artificially in a single molecule, the product is a new species having novel selectivity for a corresponding heteropolyvalent target, provided that the interaction can take place without either strain or excessive degrees of freedom, which should be possible through the use of nucleic acid linkers between the ligands. Such reagents (co-bodies) seem destined for use as hinted in proposition 6, but remain theoretical at this time [5].

The complexity of cancer appears as a barrier to devising methods of treatment [6]. We can destroy or remove many cancer cells, but not all; we can put the cancer phenotype into reverse for a while, but not forever. Likewise, Achilles seemed invulnerable until Paris learned about the most unlikely spot of all and then one well-aimed, poisoned arrow was sufficient. Perhaps its singularity will offer a parallel point of weakness for our attack on cancer. If all cancer cells in the body can be identified then all can be destroyed, which must be the gold standard for cancer treatment, notwithstanding the recent success in some cases of approaches based on phenotypic control.

Conflict of Interest Statement

The author is a Director of Hybrid Antibody Technology Ltd, a company founded to develop the co-bodies mentioned in the text and is the inventor of patent GB 2291877.

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