Contribution of Circulating Tumour Cells in Drug Development

PEER REVIEWED

Abstract
Circulating tumour cells (CTCs) are becoming very welcomed in the field of oncology, and they are also promising in the clinical field, in the area of drug development. Proof of concept that CTCs may correlate with the prognosis of cancer has been completed for many types already. Also, the CTCs may carry information that is relevant to the disease progression and behaviour and this data cannot be obtained from the primary tumour. Hence they are becoming extremely valuable in the field of drug development and evaluation of candidate medications in pharmaceutical research and industry. The ability to isolate and analyse them is already known, including the limitations and barriers. The rationale and the concept of utility of CTCs and the relevant cancer stem cell like as a subset, could offer more possible solutions and further targeted therapeutic approaches for treating cancer.

Introduction

After many decades of endeavour in drug development, today we have concluded a more rational and target-based drug development based on the identification of the proper target, and on the understanding of the precise mechanism of a disease. Especially in the disease of cancer, the progress of molecular biology and the breakthrough achievements in the physiology of cancer progression and metastases lead us to understand the importance of heterogeneity of the disease, and that our interest should focus on the proper entity which determines the disease progression and relapse. Since circulating tumour cells (CTCs) are disseminated from the primary lump and through them the relapses are generated, they become the carrier of the cancer cells population of interest. Therefore, they become very important and popular in our quest for understanding cancer development and progression, in order to point out new therapeutic targets and create new therapeutic molecules.

Physiology/Biology of CTCs and CSCs

It is well established that the primary tumour mainly starts from a multiple process of carcinogenesis that is composed of an accumulation of aberrations and abnormalities which finally spawn the malignant phenotype of an abnormal malignant cell. Then the cancerous cell, without any control of the cell division mechanism, quickly replicates at the same time as the immune system is reacting against the abnormal cells. This point is called equilibrium[1]. In contrast, the genetic instability of the cancerous cells generates heterogeneity and pleomorphy of the abnormal cells, and the best fit clones are replicated fast and are no longer detectable from the immune-surveillance. At that stage the cancerous tumour is entering the phase of immune-editing and immune-escape[2]. Many mechanisms are implicated in this process, like the HLA histocompatibility system or even the interaction between tumour cells and the immune system, through cytokines and receptors like TNF-a, or ligands PD-1, etc. When the bulk of the tumour cells are growing, the hypoxia triggers the mechanisms of neovascularisation. In this phase the tumour cells excrete factors that attract normal endothelial cells and fibroblasts that form abnormal vessels of the lymph or blood circulation towards the position of the tumour, and through them more nutrients and oxygen are supplied to the primary tumour cells. Then the sub-clone of the primary lump is able to invade the circulation and perform the process of epithelial to mesenchymal transition (EMT)[3]. The cells may circulate to the "depositor" organs, like bone marrow or liver. They may relocate there and remain in dormancy and receive the influence of several triggering factors like bone morphogenic proteins (BMPs), which additionally alter the circulating tumour cells phenotype[4]. When these disseminated circulating tumour cells arrive at the organ, the cells there have specific markers on their surface and the proteins interact between the disseminated tumour cells and the tissue of the distant organ[5, 6].

As is obvious from the previous outline of the cancer physiology, the circulating tumour cells are the intermediate entity which may include the clone of cancer cells that may initiate the growth of a tumour in a distant organ. This particular clone of cancer cells is called tumour initiating cells, or cancer stem cells like. The properties of this clone are that they are able to completely regrow a tumour when they are engrafted. It is also essential to distinguish the entities of CTCs and CSCs, which are not identical although the CTCs can include the CSCs as a subset. This particular feature makes the entity of CTCs favourable as a sample, in order to identify and isolate the CSCs in order to be analysed.

At that point it is essential to understand that the present therapeutic strategies are based on data obtained from the primary tumour sample, which may often be different compared to the disseminated tumour cells or the distant metastases. This is the major reason why the therapeutic protocols are generating such poor responses and clinical benefit in advance stages of the disease. There is a need to point out relevant samples and new markers that will determine new therapeutic options or even new molecules that may prove to be beneficial for advanced stages of the disease.

Discussions

There are several approaches to isolating the CTCs from a blood sample and preserving the viability of the cells. The difficulties exist in two main points:

- One point is the difficulty to identify all CTCs including all the necessary sub-clones without any irrelevant kinds of cell,
- The other point is to preserve the viability of the CTCs using our technique without interfering with their viability and their phenotype, while at the same time having enough material (number of cells) to perform comprehensive analysis.

In the attempts to resolve the first difficulty, there are two main strategies: one is the positive and the other is the negative selection-based methods. In the positive selection methods, we speculate that one or a few marker/s will be present on the CTCs' surface and we select the cells based only on that factor. On the negative-based selection method the strategy is to subtract the unwanted and irrelevant cells and the remaining

cells will be all the subsets of CTCs. The first strategy has the disadvantage that there are subsets of CTCs that will not express the expected membrane marker, and this particular subset may have tumour initiative properties. The second strategy is laborious and requires multi-parameter standardization, and very few combinations of techniques can achieve such an outcome (Figure 1).

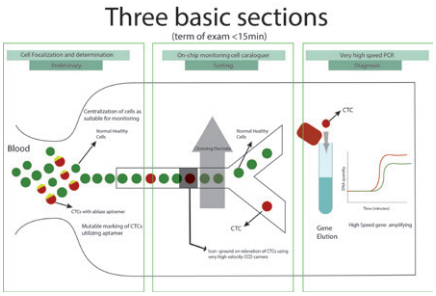

Figure 1. Circulating tumour cells detection system

The second difficulty is mainly caused by the fact that CTCs are considered as rare events. The CTCs exist in a ratio of up to 1 CTC per 10^6 WBC, and this means that there are very few cells of interest from a blood sample to perform a comprehensive analysis (Figure 2). Therefore there are attempts to "amplify" the cells of interest, either by molecular techniques like qPCR or by cell expansion. The first approach causes very easy biases since it affects the actual cell profile by the pre-amplification step and the results are altered compared to the original profile, and the second approach is difficult and requires precision and different approaches on cell expansion methodologies in order to generate repeatable results. Nevertheless, the combination of techniques can today provide possibilities to resolve all aspects of isolation, enumeration and analysis of CTCs.

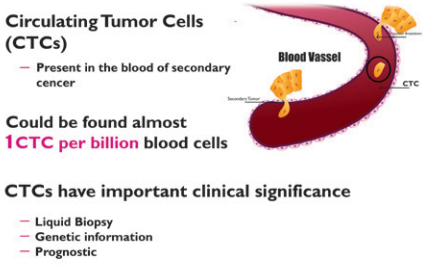

Figure 2. Circulating tumour cells

The utility of the number of CTCs has already been proven in clinical practice, since there is a strong relationship with prognostic value in several types of malignancies. Also, there is a strong prior art that supports CTC's diagnostic and therapeutic value. To be precise it is well known that the number of CTCs is related with the spread of the disease, but also the fingerprinting of the CTCs is related to the primary, but equally with the metastases of the disease. Hence the analysis of the CTCs can be a tool to identify therapeutic options for primary, as well as for metastases or recurrences (Figure 3).

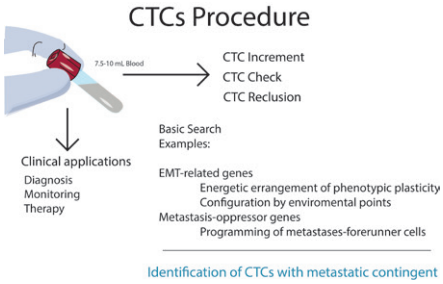

Figure 3. Circulating tumour cells

So the utility in clinical practice can be as follows:

- The concentration of CTCs can become a prognostic tool but also a method of responsiveness to a therapy
- The detection and enumeration of CTCs can be a diagnostic tool for relapses or recurrences, even before they become macroscopically present
- The analysis of CTCs (genomic, epigenetic, proteomic, etc.) can provide information about the efficacy of the proposed therapeutic strategy and the existing endogenous mechanisms of resistance (Figure 4)

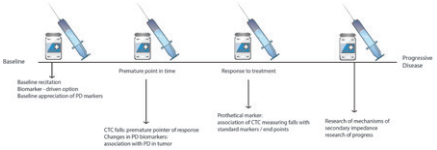

Figure 4. Analysis of the efficacy of the therapeutic strategy

Based on the same principles, the same utilities of CTCs and CSCs in a broader spectrum can be offered to the drug development sector. To be specific, the analysis of CTCs and CSCs on a genomic, as well as epigenetic and proteomic, level may reveal repeatable patterns on specific types of malignancies and in a specific stage of histological type. Since CTCs are the connective link between the primary and the recurrences they become favourable samples to be analysed in order to identify new "druggable" targets or biomarkers (Figure 5). When the repeatable epigenetic or proteomic patterns are validated, then they may have therapeutic, prognostic or diagnostic value. The main method of target validation is the technique of knocking down. When this methodology is applied and the phenotype is altered, then the target may have therapeutic value and the particular target-protein is used as primary material for drug development. According to the location of the target-protein, the selection for drug development is decided. If the location is on the cancer cell membrane or in the extracellular matrix, then it is more favourable to follow the development of therapeutic monoclonal antibodies. In that case, the protein domains are assessed according to their antigenicity and based on that the process of generation of hybridomas and production of monoclonal antibodies (MoAb)[7, 8]. If the target-protein is located intracellularly, then it is more rational to apply the process of small molecular weight organic molecule development. In that case, the protein is analysed in order to detect the active site of it and the possible ligand that bonds to that site of the protein. Then a ligand-based drug design is applied in order to generate leading compounds. If there is no ligand available but only the active site is known, then a structure-based drug design or a fragment-based strategy is utilised in order to generate leading compounds. If no active site is known, then a *de novo* drug design is used, in which the homology of the target protein with other proteins or similar proteins of other species is used, so that a ligand or a possible active site will be determined. When all *in silico* leading compounds are available, then a combinatorial chemistry is applied for synthesis which is then optimised according to the biochemical or biological assays of assessment. Then the candidate molecules may enter the *in vivo* studies on animals and then proceed to clinical trials.

In the stage of clinical trials, it is well known that in Phase I, the toxicity and the tolerability are assessed, but in Phase II and III the efficacy of the candidate is assessed and many recruits are required. At that point, the CTCs can again become a useful "tool", either to primarily prove the efficacy of the candidate medication

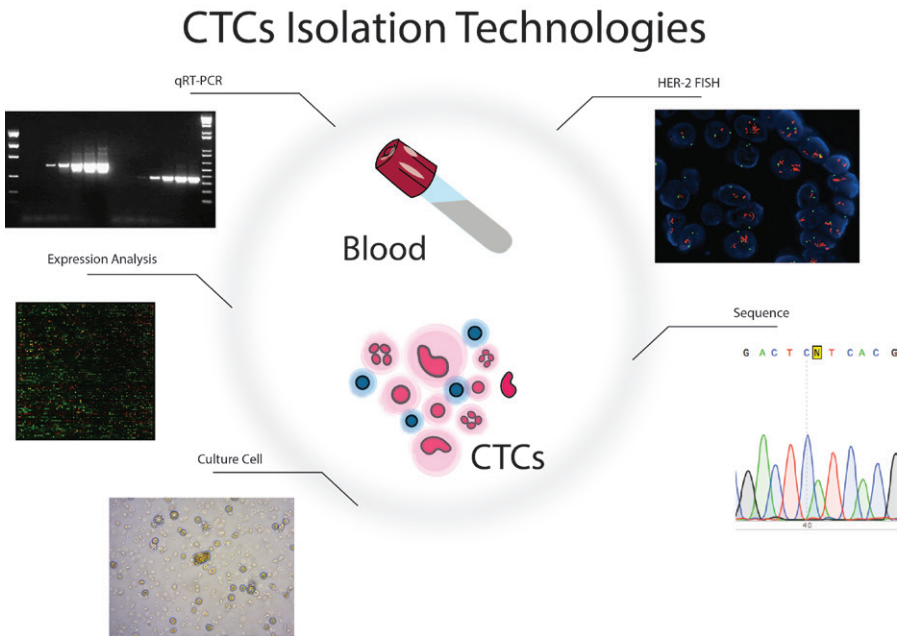

Figure 5. Circulating tumour cells technologies

by measuring the concentration of CTCs before and after application, or to be used as an assay to select the subset of patients for which the candidate drug may be beneficial, and exclude the rest[9,10]. By that approach, the CTCs may avoid unnecessary expense on failed trials. Additionally, by selecting the set of patients that will benefit from a candidate drug, the rate of successful clinical trials is increased. Finally, the candidate drug may also be connected with a diagnostic parameter in order to increase the spectrum of personalised medicine in oncology, since the beneficial subset will be detected.

Conclusions

All the above support the value of CTCs in clinical practice as well as in drug development. With further assessment and analysis of CTCs, the concept of using them as one of the major tools for a personalised approach in cancer treatment is becoming more and more established. The need for more druggable targets as well as new candidate drugs is forcing the more frequent implementation of CTC analysis in drug development and more widely in cancer treatment.

References

1. Bathia A, Kumar Y. Cancer-Immune Equilibrium: Questions unanswered, Cancer Micro-environment. 4, 209-217 (2011)
2. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases — elimination, equilibrium and escape, Curr Opin Immunol. 27, 16–25 (2014)
3. Dhruve SJ, Jared BC, Alex B, Raj M, Meena J-U. Molecular Pathways Mediating Metastases to the Brain via Epithelial-to-Mesenchymal Transition: Genes, Proteins, and Functional Analysis. Anticancer Research. 36, 523-532 (2016)
4. Kobayashi A, Okuda H, Xing F, Pandey PR, Watabe M, Hirota S, et al. Bone morphogenetic protein 7 in dormancy and metastasis of prostate cancer stem-like cells in bone. J Exp Med. 208, 2641–2655, (2011)
5. Lam H-M, Vessella RL, Morrissey C. The Role of the Microenvironment – Dormant Prostate Disseminated Tumor Cells in the Bone Marrow. Drug Discov Today Technol. 11, 41–47 (2014 Mar)
6. Rahman M, Mohammed S. Breast cancer metastasis and the lymphatic system, Oncol Lett. 10(3), 1233–1239. (2015 Sep)
7. Dillman RO, Hendrix CS. Unique aspects of supportive care using monoclonal antibodies in cancer treatment. Support Cancer Ther. 1(1), 38-48 (2003 Oct 1)
8. El Miedany Y. MABS: targeted therapy tailored to the patient's need. Br J Nurs. 24(16 Suppl 1). S4-13 (2015 Sep)
9. Huang SK, Hoon DS. Liquid biopsy utility for the surveillance of cutaneous malignant melanoma patients. Mol Oncol. 10(3), 450-63 (2016 Mar)
10. Malara NM, Givigliano F, Trunzo V, Macrina L, Raso C, Amodio N, Aprigliano S, Minniti AM, Russo V, Roveda L, Coluccio ML, Fini M, Voci P, Prati U, Di Fabrizio E, Mollace V. In vitro expansion of tumour cells derived from blood and tumour tissue is useful to redefine personalized treatment in non-small cell lung cancer patients. J Biol Regul Homeost Agents. 28(4), 717-31 (2014 Oct-Dec)

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