CIRCULATING TUMOUR CELLS (CTCs) - A SUMMARY

Circulating Tumour Cells are emerging as a vital tool in the diagnosis and monitoring of malignant disease [1-3].

“It is undeniable that CTCs have enormous research potential for individualised medicine in the future.” [3]

The purpose of this paper is to summarise the literature currently available about Circulating Tumour Cells (CTCs). Topics covered are as follows: the definition of CTCs and their unequivocal role in malignant disease and how the analysis of CTCs may assist in individualising the diagnosis, aid in therapeutic choices and enable monitoring of the progression of malignant disease.

Research Method: The phrase ‘Circulating Tumour Cells’ produces well over a thousand search results in the databases of PubMed, Cinahl, Proquest, and Google Scholar. Based on the topics mentioned above, 350 research articles published predominantly in the last seven years were extrapolated from the search. Of these 150 are review articles. 65 of the review articles on Circulating Tumour Cells (CTCs) have been published in the last two years (2008-2010). Thirty of these articles have been cited in a comprehensive document that is currently being reviewed and edited. The other half of the 2008-2010 reviews are yet to be included in the article. This paper is a summary of the document, and is intended for medical practitioners interested in gaining an understanding of Circulating Tumour Cells. The estimated time of final copy is July 2010.

CTCs and their role in malignant disease

Circulating Tumour Cells (CTCs) sparked scientific interest over fifty years ago and their detection and analysis is proving to be an invaluable tool in the individualisation of cancer diagnosis and treatment [3].

It is very well established that Circulating Tumour Cells are absolutely essential for the establishment of metastases: they function as the single haematological route of malignant neoplasias and metastases cannot occur without them [1]. In fact, ‘metastatic insufficiency’ is officially defined as the elimination of CTCs [4]. Regardless of their critical role in the metastatic cascade and despite the need for their detection and analysis as a widespread tool used in cancer management [5-7], a definition of CTCs has yet to enter a medical dictionary.

CTCs are a subpopulation of tumour cells derived from the primary cancer site that have:

- detached from the primary tumour mass [8]
- adopted genetic mutations that enabled migration through the basement membrane (if the tumour is of epithelial origin) and the extracellular matrix [4, 9],

- de-differentiated or undergone Epithelial-Mesenchymal Transition (carcinoma derived cells only) [1, 4],
- entered into the peripheral blood stream where they circulate as tumour cells with metastatic potential – this is the point at which they are termed 'Circulating Tumour Cells.' [1, 10],
- have the potential to disseminate and proliferate as a metastatic lesion[1, 4],
- Can stimulate angiogenesis . [1, 10],
- have stem-cell like properties (see below) [1, 2, 11].

Survival of CTCs in the circulation requires evasion of anoikis and of the immune system. There are complex mechanisms present in CTCs that allow for this prevarication to occur [9, 12, 13]. When the intercellular signaling is appropriate, CTCs extravase from the circulation, disseminate in a tissue foreign to that of the primary lesion, and proliferate in the 'permissive' organ [1, 3, 5, 10, 14]. This proliferating mass forms a secondary cancer at a site foreign to that of the primary cancer [1].

Stephen Paget's well recognised 'seed and soil' hypothesis states that metastases exhibit tropism, i.e. the organ site wherein they disseminate and form a secondary tumour is not random [5, 14]. The organ site of a metastasis is the 'soil' which is absolutely biologically the ideal place for a specific 'seed' (CTC) to grow [1]. Both the CTC (seed) and the organ site (soil), which will harbour the metastases, have biomarkers that specifically recognise and interact with each other [1] . Together they facilitate the development of the environment necessary for a metastatic lesion to develop and thrive [1, 3, 5, 10, 14]. CTCs have adopted genetic mutations that equip them to respond to local growth factors and stimulate neovascularisation in the microenvironment of new site. [5, 14] [4]. These biological markers on CTCs may differ entirely from the markers of the bulk of primary cancer cells [1, 11].

Heterogenicity of Tumour Cells: Understanding the heterogeneous nature of tumour cells is necessary in order to fully appreciate the critical role CTCs play in the formation of metastases [15]. A vast number of the CTC characteristics are yet to be determined, however, it is known that CTCs are likely to have heterogeneous biomarkers to that of the parent tissue and other subpopulations of the primary tumour [1, 16]. Common CTC properties that identify them as heterogeneous to other primary cancer cells are their increased invasiveness, their heightened resistance to threat, and their biological likeness to stem or progenitor cells [1, 4, 6].

The heterogeneous nature of tumour has the following consequences:

- Classification and morphological analysis of tumour cells from a surgical biopsy may differ to the character of the tumour's CTCs [1, 11].

- The majority of the cells of the biopsy will not have initiating capacity and therefore may be less relevant in terms of diagnosis and treatment [1].

- CTCs have the potential to behave totally differently to the original primary cancer cells and respond to entirely different treatments [1, 11]

CTCs and Tumour Initiating Cells (TICs)

(*N.B There is confusing terminology existing in the literature about tumour stem cells. Tumour cells that have progenitor/stem cell characteristics and are responsible for tumour progression are called Tumour Initiating Cells (TICs) [15]. They are known colloquially as 'Cancer Stem Cells' (CSCs) [17])

CTCs share similar genotypic and phenotypic characteristics with Tumour Initiating Cells (TICs) [1, 6]. CTCs have the capacity to self-renew, to divide asymmetrically, for genetic adaptation, to accumulate mutations [4]. They have the ability to sustain tumour genesis and growth, and to initiate tumours with multiple descendent lines. [2, 11, 18]. CTCs may circulate as non-proliferating tumour cells, potentiating their resistance to chemotherapy [19, 20]. They can transition from this non-proliferating pluripotent-progenitor cell phenotype into a proliferating cell upon dissemination [21].

The similarities that CTCs have to cancer stem cells may explain the eventual relapse of disease in a patient previously considered to be in remission following primary therapy [6, 15, 22].

The sub-population of neoplastic cells that have stem cell properties are known to:

- be responsible for tumour progression [15]

- have unique biomarkers that may correspond to radio- and chemotherapy resistant mechanisms [23]

- derived from and regulated by both genetic and epigenetic programs [24]

If therapy is to be targeted toward cells responsible for tumour progression, these epigenetic determinants of mutations need to be considered [24].

Cancer treatments may be unsuccessful if they fail to target the specific minority subpopulation of tumour cells that have capacity for invasion and tumour initiation [15]. These populations are an absolutely essential target for therapy and if metastatic disease is to be prevented. [15, 25]

What can CTCs tell us about the patient's malignancy?

"CTCs have a wealth of clinical information in the evaluation of tumour progression, prediction of long term prognosis, identification of patients who are likely to respond to treatment of curative intent, and assessment of likelihood of recurrence" [4]

The identification and analysis of CTCs is emerging as an essential clinical tool in the diagnosis of malignancy, and in the monitoring of disease progression and effect of cancer treatment [1, 3, 26, 27].

CTC detection and analysis is a valuable tool in the management of cancer because it enables the following information to be realised:

1.Evaluation of tumour progression in real-time.

Analysis of CTCs enriched from the peripheral blood of patients with advanced or metastasising cancer represents the real-time biopsy that has been up until this point impossible without surgical intervention [18][11]. Detection of CTCs in the peripheral circulation of cancer patients indicates the presence of metastatic disease [1, 4, 11]. Due to the ease of sample collection, it is possible to monitor tumour progression and stage, and assist in determining the success of cancer treatment [5].

CTC count in the peripheral blood of a patient is indicative of tumour stage, tumour progression, and success of treatment [1]. The difference in CTC count between two samples, taken prior to and following surgery or cancer therapy, can inform the practitioner of the success of the treatment [5]. CTC count falls significantly with the regressing of disease, and similarly CTC count rises with the advancement of the malignancy [4, 5].

The CTC count is indicative of tumour stage [3]. The numerical value which determines how advanced the cancer is will differ across the various types of malignant neoplasias, and their comparative averages have already been determined [4]. For example, more than 5 CTCs per 7.5ml of peripheral blood is considered to be a progressive disease.

2.Prediction of long-term prognosis

The presence of CTCs in the peripheral circulation has been confirmed as an independent prognostic indicator [1, 5]. CTC detection is predictive of clinical outcome and overall survival rate in multiple malignancies. [1, 5] The prognostic significance of CTCs relates to time to disease progression and to the prediction of recurrence, even after therapy of curative intent [1, 4, 14].

CTC detection in the blood may override the standard prognostic indicators [2, 4]. Specifically, detection and analysis of CTCs may be a more accurate predictor of clinical outcome in terms of Overall Survival than standard prognostic indicators [2]. Multivariate analysis has shown that CTC count is an independent prognostic indicator irrespective of other variables [1, 5, 6].

The presence of CTCs at time of diagnosis is an indicator of whether adjuvant chemotherapy is needed in early stage cancer patients [3]. Due to the similarity between Cancer Stem Cells and CTCs, (i.e their characteristics of longevity, ability of tumour initiation, self renewing and proliferative capacity), the presence of CTCs at the time of diagnosis and treatment, may explain the eventual relapse of disease in patients who have previously been 'in remission' after primary therapy [6].

3.Identification of patients who are likely to respond to treatment of curative intent

It is difficult to predict the biological fate of the cancer from biopsies obtained from the primary cancer [28]. A significant number of patients experience metastatic disease following primary therapy due to the treatment's inability to target the more aggressive metastasising population [3].

The biological fate of malignancies is determinable through the detection and bio-characterisation of CTCs [18, 28]. Chemosensitivity testing on the isolated CTC population can identify treatments that are likely to instigate the apoptosis of metastasising cells [3]. It follows that the benefits of CTC analysis and testing will have implications in clinical decision-making, making it possible to individualise diagnosis and treatment plans [3, 6, 29].

4.Assessment of likelihood of recurrence.

CTC detection and analysis makes it possible to assess the risk of disease recurrence after therapy of curative intent [1, 4]. CTC count in the peripheral circulation before both surgery and chemotherapy or other treatment is the marker that can independently predict the early recurrence in patients with cancer [14]. Novel enrichment and molecular analytic techniques have made it possible to detect metastasising disease that is undetectable using conventional imaging techniques. [4]

The detection and isolation of CTCs

Circulating Tumour Cells (CTCs) are rare events in the peripheral circulation of cancer patients with malignant disease [7]. They can be reliably detected, isolated, cultured and analysed using immunocytochemical and biomolecular techniques [1, 2, 7, 16].

When used in isolation, each of the available detection methods have their advantages and pitfalls [1]. There is yet to be a standardised CTC-enrichment technique, however, the FDA has approved the Veridex (Johnson & Johnson) CellSearch device for CTC detection in breast cancer patients only [1,4]. CellSearch is known for it's high specificity, but poor sensitivity [4]. Numerous studies indicate that using a combination of the more recent physical and immunochemical techniques overcomes the disadvantages each method may have when used on their own [1, 2, 6, 7, 16].

CTCs either express or, (given certain conditions), have the potential to express renegade proteins that are associated with the robustness of malignant tumours [30]. The earlier that cells with

tumour initiation capacity are detected and analysed, the sooner an individualised treatment design is possible. [15, 31] The identification of both surface and intracellular markers that indicate metastatic progression are they key to detecting the CTCs in the blood.

As yet it is too complex to detect and isolate tumour stem-cells within a tumour mass due to the lack of identifiable stem cell markers [15]. The detection of CTCs however overcomes this problem: their significance lies in their similarity to tumour stem cells, and they are easily isolated from the peripheral circulation [1, 3].

Methods of enrichment may involve one or a combination of the following:

PHYSICAL:

- Centrifugation: isolating CTCs based on their gradient-density [1, 4, 16]
- ISET: 'Isolation by Size of Epithelial Tumour cell' [1, 3, 4, 6, 29]
- Isolation by other morphological characteristics unique to CTCs [4].

IMMUNOCHEMICAL

- ICE – 'Immunomagnetic Cell Enrichment' enriches CTCs via either positive or negative selection. ICE involves antibodies bound to magnetic beads that are selective to CTC markers. Isolating the antibody-selected cell complex from the blood occurs due to exposure to a magnetic field [1, 4]. CellSearch isolates CTCs via positive selection by utilising ICE and histological staining of EPCAM markers [4].

Biomolecular analysis of CTCs

RT-PCR:

- Reverse Transcriptidase Polymerase Chain Reaction detects genetic mutations in the DNA of CTCs. Primers or probes are designed which base-pair with the specific gene or chromosomal sequence (mutation) of interest, thereby identifying their presence. Multiple sequences (in fact the entire genomic sequence) can be analysed simultaneously [1].

DNA MICROARRAY:

- DNA microarrays enable the identification of genes, determines the active expression of genomic sequences, and detects oncogenic mutations/polymorphisms present in the nucleic acids of any cell. [32] The process makes biochemical calculations of the mRNA that is expressed in cells, hence revealing the cell's molecular biology. DNA-microarrays can analyse multiple genes

simultaneously and have revolutionary diagnostic potential. [31, 33]

FLOW CYTOMETRY:

- Flow Cytomertry examines the biomolecular footprint of cells. In a nutshell, a cell is tagged for specific constituents and exposed to a laser beam of light. The presence of proteins and sub-cellular molecules in/on the cell will cause the light to fragment in a pattern that, in turn, identifies their existence. The patterns created by the scattering of light can be detected and analysed [34-38].

Chemosensitivity testing of CTCs

Chemosensitivity testing of CTCs is a diagnostic tool that enables individualised tailoring of cancer treatment [3]. Chemosensitivity testing involves exposing monocultures of enriched CTCs to each available cancer therapy agent and then analysing the cell's resistance or sensitivity to the treatment. The pathologist can observe sensitivity to treatment by calculating the extent to which a chemotherapy agent induces apoptosis of cultured cells [25]. The pathologist can also observe resistant mechanisms present in the culture of CTCs by observing the extent to which the cells maintain an active cycle despite exposure to the agent [25]. Recent studies that clarify the prospect of individualised cancer treatment through chemosensitivity testing all affirm the importance of testing resistant mechanisms in cells of tumour initiating capacity (TICs and CTCs) [3, 25]. These biomolecular markers on TICs and CTCs may be responsible for the failure of primary therapy and therefore are a promising target of individualised anti-cancer therapy [3, 17, 25, 29].

The significance of CTCs in terms of diagnosis and treatment

"It is undeniable that CTCs have enormous research potential for individualised medicine in the future." [3]

Molecular diagnostics hold great promise for individualised diagnosis of cancer [15]. It is possible not only to detect defunct proteins that regulate the cell cycle but also to scan the entire genome of metastasising cells and detect the genes associated with cancer progression prior to them even being transcribed or expressed [2, 29]. Molecular technology also allows the detection and testing of resistant or chemosensitive mechanisms existing or dormant within the tumour cell [39]. Such knowledge of the biology of a patient's caner allows clinicians to select effective targeted therapies, to monitor the effects of treatment in real-time, and to adapt treatment according to new mutations or protein expression that may have arisen [2, 29]. Detection of these mechanisms is highly valuable in effective cancer management. [3, 18].

A major factor contributing to the possibility of individualised diagnosis through detection and analysis of CTCs is simply the ease of sample collection and accessibility of the cells [3].

Traditionally, clinicians have had to obtain a tissue sample that needs preservation in formalin and fixing in paraffin in order to analyse cancer cells [1]. Analysis of cancer cells isolated from the peripheral circulation overcomes this hassle as well as providing a continuous source of DNA, being free of selection bias, being instantaneous, less expensive and far less invasive than a biopsy surgically removed from a solid tumour [4, 15].

Individualised treatment arises from the possibility of assessing treatment efficacy, assessing completeness of surgery, and monitoring the changing molecular biology of heterogeneous subpopulations of cancer cells [40]. Heterogeneous mutations and protein expression can be detected through highly sensitive methods of analysis of CTCs, deeming CTCs potentially central to the tailoring of cancer therapy [3, 4]

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