Causes and stages of cancer

Introduction

The World Heath Organisation (2011) estimates that without intervention 84 million people will die from cancer between 2005 and 2015. In 2008 156, 723 people died in the UK alone of malignant neoplasm and 1 in 3 people will be diagnosed with the disease in their lifetime. There were 12. 7 million new cases diagnosed worldwide in the same year which led to 7. 6 million deaths. (Cancer Research UK, 2011). There are over 200 different types of cancer but lung, breast, prostate and colorectal account for over 50% of cancer cases. The percentage of deaths caused by cancer varies by region with 4% in Africa, 12% in Asia, 19% in Europe, 21% in Oceania and 23% in North America (National Cancer Institute, 2010).

Cancer is used to define the group of diseases in which extensive cellular proliferation occurs alongside the invasion of surrounding tissues. Cancer cells can spread through the body via the blood and lymph systems and ultimately cause death of multi-cellular organisms (National Cancer Institute, 2010). Cancer is caused by the accumulation of genetic mutations which leads to instability of genetic regulators and alters gene expression. Cancer represents not a single disease but a group of heterogeneous diseases that share the fundamental biological characteristics of immortalisation, invasion, genetic instability, erratic differentiation and uncontrolled proliferation (Vogelstein and Kinzler, 2008). Despite advances in detection and treatment of metastatic cancer, specifically breast, mortality rates still remain high because current therapies are limited by the emergence of therapy resistant cancer cells (Al-Hajj, et al, 2003).

It has been documented that tumorous cells possess key stem cell characteristics such as high migration, self-renewal, drug resistance and extensive differentiation which leads to the production of a heterogeneous population. Tissue specific cells are able to self-renew and produce differentiated and functional cells within an organ. These differentiated cells are short lived and are produced from a small pool of long lived stem cells which lasts throughout the organism’s lifetime (Seo, 2007). Stem cells are essential for tissue development, replacement and repair however their longevity means they are susceptible to the accumulation of genetic damage and thereby providing a growth route for cancer recurrence following treatment (Clarke, 2005). Dean, Fojo and Bates (2005) suggest that cancer stem cells can survive chemotherapy and sustain the re-growth of a malignant tumour. Therefore if cancer stem cells are present in a tumour then they must be targeted in order to achieve a cure. Prospectively identifying cancer stem cells will allow investigation of the pathways and key molecules that can be targeted to eliminate these malignant cells (Clarke & Fuller, 2006).

There have been many studies which imply the existence of a sub-population of cells within tumours termed cancer stem cells which drive tumourgenesis. This paper therefore aims to isolate and characterise different sub-populations of cancer stem cells through physiologicalstressin human and murine models (DLD-1 and CT-26 respectively). There has been extensive evidence that CD133 and CD44 are reliable cancer stem cell markers therefore it can be hypothesised that CD133 and CD44 positive cells would demonstrate resistance to chemotherapeutic agents. This statement formed

the basis of the protocol developed by Sharma (2010) where the novel technique of exposing parental cancer cells to the chemotherapy drug doxorubicin in-vitro to isolate cells resistant to drug exposure. The resulting cells will them be characterised by their ability to form spheroids and the performance of Q-PCR, immunofluorescence and western blotting to identify the presence of the CD133, CD44 and CD26 specific cancer stem cell markers. The ultimate aim is then perform microarray on parental and cancer stem cell populations to compare the difference in gene expression of the two populations.

Literature Review

On a cellular level cancer is caused by uncontrolled cell proliferation which enables abnormal growth leading to cancerous tumours. Just 5-10% of cancer cases can be attributed to genetic defects whereas the remaining cases (in order of influence) are a result of environmental factors such as diet, tobacco, infections, obesity, alcohol, radiation, stress and physical activity (Anand, et al, 2008). These factors lead to tumour growth as they induce DNA alterations or loss of the ability to repair DNA damage which deregulates standard gene expression (Vogelstein and Kinzler, 1998).

Carcinogenesis is the term used to define the creation of cancer by which normal cells are transformed into cancer cells. This is due to the accumulation of genetic mutations and the resultant misbalance in cell death and proliferation (King & Robins, 2006). Cell production is a complicated process which is kept in apoptosis via cell regulation by numerous classes of genes including oncogenes and tumour suppressor genes (Vogelstein and Kinzler, 1998). Carcinogenesis occurs when there is a genetic mutation

which upsets the normal balance between cell death and proliferation. The multi-step process is driven by the accumulation of genetic alterations which gives rise to highly malignant derivatives which have the ability to elude apoptosis, invade tissues and possess unlimited potential for replication (Hanahan and Weinberg, 2000). The inheritance of a defective gene itself is not sufficient for development of cancer. Cancer manifests from the accumulation of additional somatic mutations which occur as a result of imperfect DNA replication or DNA damage caused by environmental mutagens. Genes that, when mutated, lead to cancer predisposition normally have the function of suppressing tumourgeneis. If one allele of such gene mutates in the germ line then the cell still has the product of the wild type as a back up. If a mutation occurs in the wild type then the cell has no functional suppressor gene product remaining. The cell therefore proliferates abnormally leading to clonal expansion. Cells of proliferating clones are likely to accumulate another mutation resulting in further loss of growth control. As gradual clonal expansion takes place a tumour evolves. Oncogenes and cell suppressor cells control cell proliferation, a mutation here leads to the cells to become continually active. Caretaker genes control rates of mutation, defective caretakers therefore acquire mutations (Vogelstein and Kinzler, 1998).

The three stages of carcinogenesis are promotion, proliferation and progression. Changes in the genomes structure occur across all three of the stages of neoplasm development. Additionally changes in gene expression take place at cell promotion with selective proliferation of mutation cells. Apoptosis and cell proliferation occur at different rates but still maintain a

balance during initiation and promotion but during progression the balance alters and a malignancy arises as seen in figure 1 (Oliveira, 2007). The fundamental progression features of malignancies are invasion and metastasis and it is these traits which distinguish between normal and cancerous cells. Metastasis is characterised as the migration of cancer cells from the site of origin to a secondary point through the lymphatic system, connective tissues and blood supply. At this secondary point the cells then continue to invade and form new tumours (Hanahan, 2000).

Cancer immunoediting has been described as the conflicting action of the immune system to protect the host from cancer development through immunosurveillance and promote tumour growth by the promoting action of immunity (Smyth, Gunn and Schreiber, 2006). The interaction of the innate and adaptive anti-cancer immunity dictates the intensity of the outcome of the endogenous anti-cancer response. Stress induced molecules on tumour cells initiates the innate response and presentation and processing of tumour associates antigens leads to an adaptive response. Both of these responses can affect the tumour in different ways. The endogenous reaction could suppress tumour formation whilst at the same time exerting a selection pressure leading to the emergence of escape variants. Additionally the host’s immune response could directly promote tumour growth, invasion and metastasis via elaboration of inflammatory mediators and cytokines. There is a complex network of interactions between tumour cells, immune elements and stromal components in the microenvironment (Jinushi and Dranoff, 2007). However currently only the capability of the immune system to identify and kill cancer cells forms the basis of therapeutic strategies and

immunotherapy (Schulz, 2005). Modifications in immunotherapy protocols have been proposed to lessen the effect cancer and improve the therapeutic value of immunological approaches by targeting the elimination of cancer stem cells (Lepisto, McKolanis & Finn, 2007).

Stem cells originate from the haematopoietic tissue and can be characterised by the unlimited capacity to self renew, which is the result of increases telomere activity (Huntly & Gilliand, 2005) and the ability to terminally differentiate into one or more cell types, which is regulated by a niche signalling pathway system (Spradling, Drummond-Barbosa & Kai, 2001). The capacity of stem cells to form differentiated offspring is described in terms of their differentiation potential (Friel, Van der Sar & Mee, 2005). Totipotent cells have the ability to construct a complete organism, pluripotent cells are descendants of totipotent cells and can differentiate into almost all cells, multipotent cells differentiate into a specificfamilyof cells, oligopotent cells differentiate into lymphoid and myeloid cells and omnipotent cells are only able to produce their own cell type but have the ability of self renewal which distinguishes them from other non-stem cells (Knoepffler, Schipanski & Sorgner, 2007). The differentiation of stem cells is regulated by a niche signalling pathway system (Spradling et al, 2001). It has been suggested that cancer stem cells are displaced due to lack of heritable changes in phenotype and genetic alteration leading to an absence in cancer however when stem cells were placed in defective tissue they induced tumour growth (Clarke & Fuller, 2006). Charafe-Jauffret, Monville and Ginester (2008) clarify the existence of cancer stem cells which possess tumorgenic, self-renewal and multi-lineage differentiation abilities.

Cancer stem cells are defined as a sub-population of cells in a tumour capable of generating phenotypically assorted cells (Gao, 2007). This petite population is accountable for the relapse of tumour growth, progress and invasion after treatment (Clarke & Fuller, 2008). Although the concept that germ cells are responsible for oncogenesis has existed since 1855 the first conclusive evidence of cancer stem cell existence was that by Bonnet and Dick (1997) who isolated a CD34+/CD38– sub-population of leukemic cells which were capable of initiating tumours in NOD/SCID mice histologically similar to the donor. The cancer stem cell hypothesis states that the cancer initiating cell is a transformed tissue stem cell which retains the property of self protection through the activity of multiple drug resistant transporters. This drug resistant cell then remains at a low frequency amongst a tumour mass (Donndenberg & Donndenberg, 2005). The cancer stem cell theory points to a new era of cancer research and is expected to yield alternative cancer treatments. It is now evident that tumours include cancer stem cells which can be isolated by antigenic markers and have the potential to develop into non-adherent spheroids (Wright et al, 2008). This concept has challenged the previous hypothesis that carcinogenesis is a result of ‘ clonal evolution’ where every cell present in a tumour is capable of proliferating and forming new tumours (Max et al, 2006). Cancer stem cells have similar properties to stem cells such as similar molecular mechanisms and physiological trafficking which implies that cancer stem cells are a result of consecutive accumulated mutations in embryonic stem cells (Kucia & Ratajckzak, 2006). This is supported by the correlation seen in the signalling pathways associated with maintenance of ‘ stemness’ in embryonic cells and cancer pathways. These pathways such as JAK/STAT, Notch, MAPK/ERK,

P13k/AKT, NF-GB and Wnt are not only involved in stem cell renewal governing proliferation but also express key molecules associated with malignant phenotypes which leads to tumour growth (Dreesen & Brivanlou, 2007). It has therefore been deduced that normal stem cells are transformed into cancer stem cells via mutations in suppressor genes and oncogenses and mutations in repair genes and histone modifications (Costa, et al, 2006).

Embryonic stem cells are dependent on the specialised microenvironment in which they reside. This niche prevents tumorgenesis by supplying signals to inhibit differentiation and proliferation. Additional signals are also provided to allow stem cell promotion, self-renewal or differentiation when necessary (Scadden, 2006). Stem cells are dependent on this niche for survival alternatively cancer stem cells do not appear to be dependant on this niche in the same way. Some believe that cancer stem cells have evolved to escape the control of a localenvironmentwhereas an additional theory suggests the cells do reside in a niche which has undergone changes itself which encourages cell growth (Burness & Sipkins, 2010). Cancer stem cells are thought to evolve from an intrinsic mutation leading to self-sufficient proliferation and deregulation as the cancer cell overtakes the molecular machinery used by normal stem cells (Li & Neaves, 2006). These tumour initiating cells are the source of recurring tumours in many types of cancer (Foltz et al, 2009). Cancer stem cells make up less than 5% of a tumour and have been found in blood-borne, brain, breast, ovarian and colon cancers (National Cancer Institute, 2010). These cells are highly resistant to both chemotherapy and radiotherapy and in order to develop successful therapy it

is essential to identify the cell surface markers unique to cancer stem cells and interpret their signalling pathways, figure 2 (Foltz et al, 2009).

Figure 2: The impact of cancer stem cells on tumour growth and response therapy. A: Subset of cells within the tumour has the ability to replicate and sustain tumour growth. TA cell is suspected to be responsible for a majority of tumour growth and is susceptible to cancer therapy. Cancer stem cells give rise to identical immortal daughter cells. B: Possible outcome of targeting tumour cancer stem cells verses present cancer therapy techniques which do not affect cancer stem cells (Houghton et al, 2007).

The developmental concept of cancer denotes the presence of a hierarchy of cells within a tumour which refers to the differences of cancer cells within a tumour where all cells do not express the same antigens. Additionally functional assays show that only a specific sub-population of cells within a tumour can propagate tumour growth. It appears that cancer cells capable of tumour growth are able to self-renew as well as generate cells which cannot propagate tumour growth (Cho & Clarke, 2008). Cancer stem cells have therefore developed the ability for self-renewal and differentiation into a heterongenous population as well as the tumour related properties of uncontrolled growth and ability to form metastasis (Dalerba, et al, 2007).

The ability that cancer stem cells share with stem cells to renew has changed perspectives leading to new approaches to treating the disease (Li and Neaves, 2006). Cancer stem cells show resistance to both chemotherapy and radiotherapy making them a crucial target for treatment, it is therefore essential to identify the markers present on these cells in order to

therapeutically target them (Foltz, et al, 2009). Current therapeutic strategies attempt to target cancer stem cells and its microenvironment whereas Tang, Ang & Pervaiz (2007) identified a novel approach of targeting the reactive oxygen species in a cancer stem cell which would facilitate apoptotic death over proliferation. Additionally the development of monoclonal antibodies to recognise cancer stem cell markers would allow for more efficient destruction of these tumour forming cells (Okamoto & Perez, 2008).

The use of immunocompromised mouse model have shown to reliably capulate the molecular, biological and clinical features of the human disease. With such models defining the stages of tumour development, homogenised breeding and environmental conditions. This has therefore led to the development of the concept that plasma from genetically modified cancer models contains tumour derived proteins that may be relevant in the development of markers for human cancer (Kuick, et al, 2007). There are numerous studies which cites the use of immuno-comprised mice as vehicles for cancer stem cell isolation. Mouse models have been established as highly trusted in the development of human cancer treatment through verify the cancer stem cell extent of a tumour. This has been achieved by the opening and repetitive tumour progression in immuno-compromised SCID mice (Laurie, et al, 2007).

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Current developed methods to isolate ‘ adult’ stem cell populations includes collection of different hematopoietic cells populations staining with the

antibodies of interest and sorting by magnetic bead and/or fluorescence activated cell sorters (FACS) followed by in vivo transplant experiments. This allows for the development of understanding of fundamental hematopoietic stem cell characteristics of differentiation and the ability of the cells to give rise to others cells with the same potential for proliferation whilst still maintaining the stem cell pool Cho & Clarke, 2008). Additional characterisation of cancer stem cells can be carried out by identifying gene expression and cell markers via immunofluorescence, western blotting and Q-PCR. It has been stated that an immature cell population can be characterised by surface markers CD34+ and CD38+ in AML and that these markers suggest the ability of initiating tumour development (Bonnet & Dick 1997). Furthermore the use of a low-adherent growth environment can be used to produce spheroids from tumorgenic cells as a basis of isolation of cancer stem cells (Grange, et al, 2008) As it has been identified that a small minority of cells present in a tumour has the ability to form new tumours. It is therefore possible to distinguish between tumorgenic and non-tumorgenic cells based on the cell surface marker expression. Al-Hajj, et al (2003) were able to isolate breast cancer cells expressing CD44+ and CD24– lineage in as few as 100 cells with tumorgenic capabilities and identified hundreds of thousands of cells with a different phenotype which failed to form new tumours. Furthermore passaging the CD44+ and CD24– lineage led to the creation of phenotypically diverse populations. Sherman, et al (2011) also identified CD133 expression correlated with prognosis of oligodendroglial and astrocytic tumors and here immunofluorescence provided an effective and reproducible assay for identifying markers present in cancer stem cells. Fundamentally cancer is resistant to treatment because malignant cells

survive chemotherapy, CD133 positive cancer stem cells display strong compatibility with tumours resistant to chemotherapy (Liu, et al, 2006).

The use of immunofluorescence to characterise cancer stem cells has been used in a great deal of cases especially where total cell count is reduced

CD44+ and CD24+ can be used for markers of colorectal cancer stem cells. CD44/CD24 cells are enriched for spheroid colonies and can reform all four CD44/CD24 subpopulations (Yeung, Wilding & Bodmer, 2009). Additionally a population of CD26+ cells present in a sub-population of colorectal cancer stem cells led to the development of distant metastasis when injected into a mouse cecal wall. These CD26+ cells were also associated with enhanced invasiveness and chemoresistance (Pang, et al, 2010). Lgr-5 has also been identified as a key marker expressed in cancer stem cells of colorectal cancer associated with the activation of the Wnt signalling pathway which plays a key role in cancer development (Takahashi, et al, 2010).

The first report indicating the difference in gene expression of cancer cells exhibiting cancer stem cell properties and those which did not was published in 2007 by Seo, et al. Amongst the 61 differently expressed genes 12 genes were considered up-regulated in the sub-population whereas 49 were downgraded validation of these gene expressions was validated using quantitative real time reverse transcriptase PCR. It was found that genes related to drug resistance such as AKR1C1/C2 and NR0B1, or cancer metastasis (TM4SF1) were up-regulated. Further more the up-regulated gene ABCG2 could be of use as an indicator for sorting. AKR1C has been identified as a catalyst of metabolic reduction and either activates or inactivates

several xenobiotics. The public database (Gene expression Omnibus) has shown significant up-regulation in expression of AKR1C1 in smokers.

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