

Deep Insight Section

Genetic instability in cancer

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Cancer is a complex disease, with multiple genes in diverse pathways involved in its initiation, progression, invasion and metastasis. In fact, it is widely accepted that the sequential accumulation of mutations that activate oncogenes and disrupt tumour suppressor genes, combined with multiple cycles of clonal selection and evolution facilitate the process of carcinogenesis. It has been estimated that disruption of about six cellular processes are required for transformation (Hanahan and Weinberg, 2000). However, a recent comprehensive sequence evaluation of colon and breast cancer genomes hints that this number may be even higher (9 for breast, 12 for colon) than previously estimated (Sjoblom et al., 2006). If this model holds true then the rate-limiting step in the process of carcinogenesis would be the rate at which new mutations occur and any factor that influences this rate should have an effect on the rate of carcinogenesis. Genetic instability refers to a set of events capable of causing unscheduled alterations, either of a temporary or permanent nature, within the genome. This term encompasses diverse genetic changes, which can be classified in a variety of ways. For simplicity we will categorize them into two major groups, instability occurring at the chromosomal level and at the nucleotide level. Instability at the nucleotide level occurs due to faulty DNA repair pathways such as base excision repair and nucleotide excision repair and includes instability of microsatellite repeat sequences (MSI) caused by defects in the mismatch repair pathway. The second form or chromosomal instability (CIN), defines the existence of accelerated rate of chromosomal alterations, which result in gains or losses of whole chromosomes as well as inversions, deletions, duplications and translocations of large chromosomal segments. Aneuploidy, which refers to an abnormal karyotype is a hallmark of many cancer cells and is thought to develop as a result of CIN. The observation that cancer cells harbour an abnormal number of chromosomes was made almost a century ago (Boveri,

1914; von Hansemann, 1890) since then we have come a long way in understanding the causes behind this type of instability. To date several pathways and processes have been implicated in CIN including :

- a) pathways involved in telomere and centromere stability,
- b) cell cycle checkpoint pathways and kinases,
- c) pathways regulating diverse proteins via post-translational modifications,
- d) sister chromatid cohesion and chromosome segregation, and
- e) centrosome duplication (Jefford and Irmingier-Finger, 2006).

Genetic instability is a very broad topic that encompasses varied fields of biology. Hence, in this article we will focus on nucleotide instability including microsatellite instability; the role of epigenetic modifications, telomeres and the environment in genetic instability; and the role of genetic instability in cancer stem cells. For further details on chromosomal instability please refer to the Deep Insight article titled Chromosomal instability by David Gisselsson.

DNA repair defects

Cells are exposed to many damaging insults capable of causing aberrations in DNA. These include environmental insults such as ultraviolet (UV) light, X-rays and genotoxic chemicals, as well as the by-products of endogenous processes such as reactive oxygen species (ROS) and lipid peroxides. In addition, some chemical bonds in DNA tend to spontaneously break down under physiologic conditions, such as when spontaneous hydrolysis of nucleotides occurs resulting in abasic sites (Hoeijmakers, 2001). In order to repair these errors and restore the integrity of the genome, the cell has in place a range of overlapping DNA repair networks. Some of the best evidence for the role of genetic instability in tumorigenesis comes from examples where mutations that cause defects in dna repair mechanisms lead to syndromes of cancer

susceptibility. Some of the common examples studied to date will be discussed below.

Mismatch Repair and Microsatellite Instability

Mismatch repair (MMR) has a central role in maintaining genomic stability by repairing DNA replication errors and inhibiting recombination between homologous sequences (Bellacosa, 2001). It is a post-replicative mechanism capable of eliminating base-base mismatches and insertion/deletion loops that arise during DNA synthesis. In the mammalian MMR system two heterodimeric complexes recognize mispaired bases; the hMSH2-hMSH3 (MutSs) complex, which preferentially recognizes insertion/deletion loops; and the hMSH2-hMSH6 (MutSa) complex, which recognizes both base-base mispairs and insertion/ deletion loops. Two other proteins, hMLH1 and hPMS2, form a heterodimer (MutLa) that is then able to bind to the previously mentioned hMSH2 heterodimers. This complex is thought to interact with and recruit other proteins required for the repair process including Exo1, PCNA, RPA and Polg. In addition, a recent report demonstrated that MutLa is a latent endonuclease that is activated in the presence of a mismatch, MutSa, RFC, PCNA and ATP (Kadyrov et al., 2006). hMLH1 has been shown to form two other heterodimers, MutLs and MutLg, with the hPMS1 and hMLH3 proteins respectively. The roles of these two complexes in post-replicative error repair remains largely inconclusive, although it is believed that each could act as a "backup" for MutLa if the need arose. MMR improves the fidelity of DNA biosynthesis 100-1000 fold and reduces the error rate to one error per 1010 bases (Modrich and Lahue, 1996). Defective MMR results in microsatellite instability (MSI), characterized by the expansion or contraction of the number of tandem repeats, due to polymerase slippage at the many microsatellite loci that occur throughout the genome.

Germline mutations in the MMR genes are associated with the inherited cancer syndrome, hereditary non-polyposis colorectal cancer (HNPCC). Instability of microsatellite repeats is seen in tumours of as many as 85% of patients with HNPCC, making it a hallmark feature of this syndrome (Aaltonen et al., 1993; Aaltonen et al., 1994). HNPCC, which accounts for about 2% of all CRC cases, is one of the most common cancer predisposition syndromes. It is an autosomal dominant disorder characterized by the development of cancer in the colon as well as in extra-colonic sites including the endometrium, stomach, urinary tract, ovaries, small bowel and brain. MMR deficiency has also been shown to give rise to sporadic colorectal, endometrial and gastric cancers. Defective mismatch repair increases the likelihood of mutations in genes containing repeat sequences that regulate growth, differentiation or apoptosis. Somatic mutations of

several genes including TGFBR2, BAX, TCF4, AXIN2, and PTEN are found in MSI positive cancers.

To date there have been reports of families with individuals who have homozygous mutations in the mismatch repair genes MLH1, MSH2, MSH6 and PMS2. Such individuals develop several congenital abnormalities including haematopoietic malignancies, pediatric brain cancers, childhood leukemia, and HNPCC-related cancers and multiple cafe-au-lait spots, a common characteristic of neurofibromatosis type 1 [De Vos et al., 2004; Gallinger et al., 2004; Menko et al., 2004; Trimbath et al., 2001; Whiteside et al., 2002; De Vos et al., 2006]. This phenotype manifests in an autosomal recessive fashion, because a mutant allele is inherited from each parent. In addition, there have been reports of individuals carrying compound heterozygous PMS2 mutations who develop Turcot syndrome (De Rosa et al., 2000). This syndrome is defined by the presence of brain tumors and multiple adenomas/colorectal cancers that occur at an early age and is associated with mutations in the APC and MMR genes.

Nucleotide Excision Repair

Nucleotide excision repair (NER) has a broader specificity in that it is able to recognize lesions as diverse as disturbances in the double helix conformation that are caused by UV light, to chemical damage that gives rise to DNA cross links/bulky adducts. The NER pathway is a multi-step process and as many as 30 proteins assemble at the damaged site in a stepwise fashion (Hoogervorst et al., 2005). Individuals born with defects in the NER pathway develop a syndrome known as Xeroderma Pigmentosum (XP). Inherited defects in any one of the 7 nucleotide excision repair XPA-XPG genes as well as XPV (a non NER gene) have been implicated in this disease (Hoeijmakers, 1994). XP patients have a very high susceptibility to developing cancer in areas of skin exposed to the sun. The median age at which skin tumours arise in these patients is 8 years, compared with a average of 60 years observed in the normal population (Kraemer, 1997). In addition a subset of XP patients show neurological defects and emerging evidence appears to indicate that the immune system of XP patients is impaired due to UV exposure (Morison et al., 1985; Norris et al., 1990; Dupuy and Lafforet, 1974; Gaspari et al., 1993; Jimbo et al., 1992). This may indicate defective immune surveillance or increased susceptibility to UV-induced immunomodulation, which may contribute to the increased susceptibility to skin cancer (Hoogervorst et al., 2005). Two other syndromes have been associated with defective NER, the first being Cockayne syndrome characterized by neurological defects and sun sensitivity but no predisposition to skin cancer (Nance and Berry, 1992). The second syndrome trichothiodystrophy is defined by patients with brittle

hair caused by a sulphur deficiency, in addition to other features such as mental retardation and small stature (Itin et al., 2001; Bergmann and Egly, 2001).

Base Excision Repair

Base excision repair (BER) is mainly responsible for repairing damage induced by endogenous metabolic processes such as methylation, deamination, reactive oxygen species (ROS) and hydrolysis (Wood et al., 2001). Multiple proteins contribute to BER pathway and enable it to correct non-bulky damaged nucleotides, abasic sites as well as single-strand breaks. The process is initiated by DNA glycosylases specific for various types of damage, which recognize and cleave the N-glycosylic bond that connects the damaged base to the DNA backbone (Barnes et al., 1993). To date, 11 such DNA glycosylases have been identified in mammals (Chan et al., 2006). Reactive oxygen species can modify the C8 position of Guanine to form 7, 8-dihydro-8-oxoguanine (8-oxoG), a major product of such damage. 8-oxoG is highly mutagenic and is able base pair with adenine and cause G:C->T:A transversions (Shibutani et al., 1991). The glycosylases most commonly involved in the removal of 8-oxoG are OGG1, MYH and MTH1. It was discovered recently that biallelic inactivation of MYH can lead to an autosomal recessive form of inherited colorectal cancer known as MYH-associate polyposis (MAP) (Al-Tassan et al., 2002). This came as a surprise to many, as unlike MMR and NER, no inherited defects in these genes had been reported prior to this (Al-Tassan et al., 2002).

Role of Epigenetic Modifications in Genetic Instability

In addition to the sequence alterations and chromosomal aberrations discussed above, epigenetic modifications that affect both DNA and the associated chromatin are capable of influencing gene expression and the stability of the genome. An important point to bear in mind is that although epigenetic modifications are mitotically heritable, they are in a state of constant flux within the lifetime of an individual. The possible contribution of the best-studied epigenetic mechanisms to genetic instability will be discussed below.

Methylation in Tumourigenesis

DNA methylation or the covalent modification of the C-5 position of cytosine residues occurs primarily at the short stretches of CG dinucleotides known as CpG islands. Recent estimates suggest that there are at least 29,000 such regions in the human genome, many of which surround the 5' ends of genes (Lander et al., 2001). In bacteria, methylation is thought to have evolved as a defense against foreign DNA. On the contrary, in eukaryotes methylation is thought to play a role in regulating gene expression and in silencing repeat elements in the genome (Jacobsen, 1999). In normal cells the pattern of expression is stably

maintained following DNA replication and cell division by a maintenance enzyme, DNA methyltransferase, (DNMT1). The establishment of DNA modifications is thought to be a highly random event (Whitelaw and Whitelaw, 2006), and could be instrumental in contributing to genetic instability. This is illustrated by the example of DNMT1, which has an estimated error rate of 5%, as well as a small rate of de novo methylation (Goyal et al., 2006; Vilkaitis et al., 2005). The first epigenetic mechanism implicated in carcinogenesis was DNA hypomethylation (Feinberg and Tycko, 2004). In addition, there have been reports of age related decreases in DNA methylation levels that occur in a tissue specific manner (Feinberg and Vogelstein, 1983; Golbus et al., 1990). It is likely that these changes contribute to the age-related increase in incidence of illnesses, such as carcinogenesis and autoimmunity (Richardson, 2003). Examples of genes hypomethylated in cancer include cyclin d2 in gastric carcinoma (Oshimo et al., 2003), Ha-RAS in lung and colon cancer (Feinberg and Vogelstein, 1983) and Maspin and S100P in pancreatic cancer (Sato et al., 2004). Several studies have implicated genomic hypomethylation in the genetic instability seen in many cancers. In a recent study of colorectal carcinomas it was shown that genome-wide hypomethylation is strongly correlated with chromosomal instability (Rodriguez et al., 2006), indicating the potential role of hypomethylation in destabilizing the genome.

CpG islands commonly occur in the promoter regions, thus hypermethylation of this region has been shown to silence gene expression (Bird, 2002). This was first identified in the retinoblastoma protein (Rb) followed by promoter hypermethylation of several other tumour suppressor and cell-cycle regulatory genes (Greger et al., 1989). It is believed that hypermethylation too is an early event that may precede the neoplastic process (Mompalmer, 2003; Nephew and Huang, 2003). A prime example of the role of hypermethylation in contributing to genetic instability is hMLH1 inactivation, where promoter hypermethylation is thought to be primarily responsible for approximately 15% of sporadic colorectal cancers associated with microsatellite instability (Kane et al., 1997; Herman et al., 1998). In a study by Costello et al. (Lander et al., 2001), 1184 unselected CpG islands were screened in 98 primary human tumours using restriction landmark genomic scanning (RLGS). This study found that on average about 600 CpG islands were aberrantly methylated in tumours, indicating the potentially vast number of genes likely to be aberrantly expressed due to this mechanism.

Methylation also plays an important role in inactivating one copy of the X chromosome, so that equal gene dosage is maintained in the somatic cells of males and females (Park and Kuroda, 2001). Imprinting refers to the phenomenon by which only the maternal or paternal allele of certain genes are expressed and the second

allele is suppressed via methylation (Ferguson-Smith and Surani, 2001). Therefore demethylation of such imprinted genes can lead to a situation where both alleles are expressed (Feinberg, 2000; Feinberg et al., 2002). This has been shown to contribute to malignancies by activating a normally silent copy of the gene as in the case of IGF2 (Rainier et al., 1993). Aberrant imprinting can also silence a normally active copy of a gene involved in growth inhibition as shown with p57kip (Thompson et al., 1996). Loss of imprinting has also been shown to contribute to certain congenital syndromes such as the Beckwith-Wiedemann Syndrome, Prader-Willi Syndrome (PWS) and Angelman's Syndrome (AS) (Maher and Reik, 2000; Reik et al., 2001). Beckwith-Wiedemann syndrome occurs due to loss of imprinting on chromosome 11p, and is characterized by pre- and post-natal overgrowth syndrome, often accompanied by exomphalos and a predisposition for childhood tumours (Paulsen and Ferguson-Smith, 2001). Loss of imprinting on chromosome 15q of the paternal and maternal alleles, lead to PWS and AS respectively. PWS is characterized by mild mental retardation, short stature and obesity, while AS is characterized by ataxia, severe mental retardation accompanied by a lack of speech, hyperactivity and a predisposition for inappropriate bouts of laughter (Paulsen and Ferguson-Smith, 2001).

Histone modification

Chromatin, which consists of repeating units called nucleosomes, is the packaged form of DNA present in the eukaryotic cell. Each nucleosome consists of DNA that is wrapped tightly around a group of conserved, highly basic proteins known as histones. Histones can be covalently modified by acetylation, methylation, phosphorylation, ubiquitination and Poly-ADP ribosylation, which ultimately influence the tightness of the protein-DNA interaction and can create a code that can be recognized by chromatin remodeling complexes (Strahl and Allis, 2000; Turner, 2002). This idea of a histone code suggests that specific patterns of modifications are read like a molecular bar code, resulting in the recruitment of cellular machinery that alter the chromatin state (Cosgrove and Wolberger, 2005). The role of histone modification and chromatin remodeling in the carcinogenic process is a rapidly evolving field. To date histone acetylation and methylation have been implicated in cancer.

It is the interplay between histone acetylases (HATs) and histone deacetylases (HDACs) that determine the precise balance of acetylation within the nucleus. Abnormal HDAC activity has been commonly observed in haematological malignancies (Espino et al., 2005). Studies done in these cancers have shown that fusion proteins such as RAR-PML and RAR-PLZF can recruit HDACs, which in turn lead to aberrant transcriptional repression that halts differentiation (de

Ruijter et al., 2003; Hong et al., 1997). It has been proposed that a dynamic relationship exists between histone modifications, chromatin structure and DNA methylation (Szyf et al., 2004; Ting et al., 2004). For example it has been shown that histone acetylation and gene activation, results in DNA demethylation (Szyf et al., 2004), while the opposite situation where low steady state level of histone acetylation and methylation, results in the recruitment of DNMT1 and DNA methylation of regulatory regions (Espino et al., 2005). Thus, it is mechanistically possible that skewed regulation of this inter-relationship could lead to genetic instability.

The role of the environment in genetic instability

Despite the many checkpoints and repair processes the cell has in place to prevent the occurrence and propagation of errors, genetic instability is a widespread phenomenon observed in many cancers. Thus, it appears likely that the environment in which these cancers arise somehow selects for and facilitates the clonal expansion of cells that show instability in their genome. This point is supported by the observation that colorectal tumours, which show an MSI or CIN phenotype exclusively, are located in anatomically distinct regions. MSI tumours are localized in the proximal section of the intestine, while CIN tumours are more frequently seen in the distal colon and rectum (Lengauer et al., 1998; Lindblom, 2001). This review will therefore briefly summarize what is currently known about the role of the macroenvironment, specifically dietary factors and the microenvironment, specifically hypoxia in the development of genetic instability.

It is possible that environmental agents are able to instigate the process of instability, as illustrated by work done in colorectal carcinogenesis. Heterocyclic amines (HAA) are carcinogens that are a common product of cooking beef, pork, poultry and fish at high temperatures. A study by Wu et al., demonstrated that patients with MSI positive cancers had significantly higher dietary exposure to heterocyclic amines, as determined by the preference for well-done meat and the frequent use of techniques that produces HAA (Wu et al., 2001). 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine or PhIP, the most abundant heterocyclic amine in the western diet, is a bulky adduct forming agent that is able to cause a variety of cancers in experimental animals (Ghoshal et al., 1994; Layton et al., 1995; Shirai et al., 1997; Tudek et al., 1989). Another powerful rodent carcinogen is MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) (Sugimura and Terada, 1998). An alkylating agent, it is able to preferentially methylate the O6 position of deoxyguanosine residues in DNA. Gastrointestinal cells are continually exposed to both PhIP and MNNG at

varying concentrations. In a study undertaken to determine if carcinogen exposure can influence the type of instability seen in cells, it was found that cells resistant to PhIP, developed a chromosomal instability or CIN phenotype, while cells resistant to MNNG exhibited MSI and associated mismatch repair defects (Bardelli et al., 2001). This data suggests that exposure to certain dietary carcinogens, may in fact select for cancer cells with distinct types of genetic instability and vice versa (Bardelli et al., 2001).

Role of tumour microenvironment in genetic instability

The tumour microenvironment has been proposed to contribute to the increased genetic instability seen in cancer cells. Several studies have lent support to this notion, including a study that demonstrated a higher rate of genomic instability of mouse cells when grown in vivo as subcutaneous tumour implants in syngeneic mice, as measured using an EGFP reporter gene and a genomic minisatellite locus (Li et al., 2001). More specifically, hypoxia has been singled out as a major microenvironmental factor. Hypoxia, which appears to occur transiently within the tumour microenvironment, has been shown to lead to cycles of hypoxia and reoxygenation (Bindra and Glazer, 2005). This is thought to lead to DNA damage as a result of reactive oxygen species (ROS) and the enzyme superoxide dismutase. In addition to ROS leading to the formation of 8-oxoG, and accumulating evidence suggest a role for oxygen and ROS in causing single and double strand breaks (Bindra and Glazer, 2005). In addition to its ability to cause aberrations in DNA, these cycles of hypoxia and reoxygenation have been shown to affect DNA synthesis, by both interrupting this process and by leading to over-replication after reoxygenation (Bindra and Glazer, 2005; Cuvier et al., 1997; Young and Hill, 1990; Young et al., 1990). Other studies have found that it is hypoxia induced gene amplification of p-Glycoprotein that is responsible for the observed resistance to adriamycin and doxorubicin (Luk et al., 1990; Rice et al., 1987), indicating that gene amplification may also be caused by hypoxia. Furthermore, emerging evidence suggests that hypoxia can influence the integrity of the genome by impacting upon DNA repair pathways. As described above, MLH1 is one of the key genes involved in mismatch repair. It was shown that hypoxia downregulates the expression of the MLH1 gene at the transcriptional level and this was thought to occur via chromatin remodeling, as treatment with an histone deacetylase inhibitor prevented the aforementioned decrease (Mihaylova et al., 2003). It has also been demonstrated that hypoxia enriches for MMR deficient cells (Hardman et al., 2001). Thus, DNA damage, defective DNA synthesis, gene amplification and the deregulation of DNA repair pathways all appear to be mechanisms by which hypoxia contributes to genetic

instability. Little is still known about other microenvironmental factors that may lead to instability. However, it has been suggested that the tumour microenvironment may represent in mammalian cells a conserved evolutionary mechanism that increases the rate of mutation in response to cellular stresses, which preferentially gives cancer cells a survival advantage (Bindra and Glazer, 2005).

Telomeres and Genetic Instability

One mechanism that can bring about chromosomal instability (CIN) is telomere loss. Although CIN is not addressed in detail in this paper, the role of telomeres is briefly summarized to highlight the important role it may play in carcinogenesis and the implications it may have in the field of genetic instability.

Telomeres refer to the segments of DNA bound by specific proteins that cap the ends of chromosomes and in doing so acts as a buffer to prevent loss of valuable genomic sequence during replication (Hoeijmakers, 2001), as well as to prevent chromosomes fusing at the ends (Jefford and Irmingier-Finger, 2006). A RNA primer is required for the process of DNA replication. Thus, when replication proceeds from the 5'→3' direction, it leaves a stretch of unreplicated DNA at the 5' end. This leads to a gradual loss of telomeric repeats and the consequent shortening of telomeres by about 50-200 base pairs, after each round of replication (Jefford and Irmingier-Finger, 2006). A specific enzyme, telomerase, maintains the telomere length. Telomerase consists of two main components; the reverse transcriptase component (hTERT), which is only expressed in cells where telomerase activity is present; and the ribonucleoprotein moiety (hTERC/hTR), which is expressed ubiquitously in all cells. In adults, telomerase activity has been observed only in immature germ cells, certain stem/progenitor cells and in a subset of somatic cells such as human fibroblasts.

Telomerase is suppressed in the majority of somatic cells leading to the continuing telomere attrition, which leads to irreversible cell-cycle arrest known as replicative cell senescence. It has been demonstrated that primary human fibroblasts that have lost the ability to senesce, display telomere shortening and eventually enter a crisis stage that culminates in chromosome fusion, aneuploidy and cell death (Counter et al., 1992). It has been proposed that it is therefore important for cancer cells to regain the ability to maintain telomeres, in order to avoid senescence and extensive chromosome fusion during crisis (Counter et al., 1992; Harley, 1995). In fact it has been shown that about 85-90% of human cancers have reactivated telomerase and are able to maintain telomere length (Jefford and Irmingier-Finger, 2006). Interestingly cancer cells that are deficient for telomerase activity are able to maintain telomere length via a mechanism known as alternative lengthening of telomeres or ALT. It has been suggested that the ALT mechanism makes use of DNA repair

pathways and recombination to maintain telomere length (Reddel, 2003). Thus, whichever mechanism employed by the cell, it appears that maintaining telomere length is critical for tumorigenesis and cellular immortalization (Jefford and Irminger-Finger, 2006). Telomere maintenance is also required for chromosomal instability. Given that cancer cells inevitably display properties of telomere maintenance and genetic instability, it has been proposed that telomere loss could be either a cause or a consequence of genetic instability (Jefford and Irminger-Finger, 2006), or perhaps be involved in both.

However, conflicting with this view is the observation that the telomeres of invasive human cancers are often shorter than their normal counterparts (de Lange, 1995). Studies in telomerase deficient mice (mTERC^{-/-}) provided a plausible explanation to this paradox (Ju and Rudolph, 2006). In these mice telomere shortening induced chromosome instability and in doing so increased the rate of tumour initiation (Rudolph et al., 2001). At the same time it was seen that telomere loss can inhibit tumour progression and the development of macroscopically advanced tumours (Rudolph et al., 2001; Gonzalez-Suarez et al., 2000; Greenberg et al., 1999; Rudolph et al., 1999). This indicates that the timing at which the telomeres shortening occurs plays a crucial role in cancer development (Meeker et al., 2004). In fact it was found that 88.6% of precursor lesions known as intraepithelial neoplasia lesions display shortening of telomeres (Meeker et al., 2004).

Cancer Stem Cells and Genetic instability

The stem cell model of carcinogenesis has been rapidly growing in popularity. The American Association for Cancer Research Stem Cell Workshop defined a cancer stem cell as a cell within the tumour that possesses the capacity to self-renew, and in doing so gives rise to the heterogeneous lineages that comprise the tumour. Cancer cells may arise therefore from tissue stem cells that have acquired mutations that render them cancerous, or it may be a more differentiated i.e. progenitor cell that may have "re-acquired" stem cell like properties due to mutations (Clarke et al., 2006). Either scenario is different from the widely accepted stochastic model of carcinogenesis. Cancer stem cells or cancer initiating cells have been identified to date in acute myelogenous leukemia (Bonnet and Dick, 1997), breast tumours (Al-Hajj et al., 2003), brain tumours (Singh et al., 2003; Singh et al., 2004) and most recently in a subset of colon tumours (O'Brien et al., 2006). The discovery of the existence of cancer initiating cells raises some very important questions regarding whether genetic instability exists within these cells and what role if any it plays in these cells.

There is an increased likelihood that exogenous and endogenous environmental agents cause a greater

degree of genetic and epigenetic changes in stem cells; as opposed to their differentiated counterparts, who by their very definition have shorter life spans. This is a fairly novel field, and much more research needs to be undertaken to determine the relationship between genetic instability and cancer stem cells. However some preliminary evidence comes from work done in haematological malignancies and telomere instability. Haematological neoplasia can be divided in to three stages, pre-malignant, chronic and acute, with the last being the most advanced stage. Telomere loss was shown to be rapid during the progression of chronic myeloid leukemia, in fact patients in the late chronic phase had shorter telomeres than those in early chronic phase (Brummendorf et al., 2001). In addition patients with pre-malignant disease with shorter telomeres had more cytogenetic abnormalities (Ohyashiki et al., 1999) and a poorer prognosis with increased rates of leukemic transformation (Sieglova et al., 2004). These observations suggest that shortening telomeres can bring about genetic instability in cancer stem cells, which is further supported by the observation that telomere shortening occurs very early in carcinogenic cascade, indicating the likelihood that this process occurs in cancer stem cells. Additionally, progression of pre-malignant disease to acute stage was shown to correlate with telomerase activation (Ohyashiki et al., 2001). Together these observation implicate telomere attrition and telomerase reactivation as risk factors for the malignant transformation of stem cells (Ju and Rudolph, 2006). On a separate note, loss of heterozygosity of cancer related genes in mammary stem cells have been shown to contribute to genetic instability in progeny cells and result in subsequent breast cancer development (Al-Hajj et al., 2003; Deng et al., 1996; Smith and Boulanger, 2002; Nguyen and Ravid, 2006). This observation also supports the notion that the theories of genetic instability and cancer stem cells are not mutually exclusive.

Summary and Conclusion

It is well documented that the sequential accumulation of mutations in tumour suppressors and oncogenes are required for the process of tumorigenesis to proceed. Any event(s) that accelerates the spontaneous rate of alterations in the cells supports this process, illustrated by the prevalence of genetic instability in cancer cells. DNA repair processes play a critical role in repairing damaged DNA, and in ensuring faithful transmission of genetic material. Thus, it comes as no surprise that inherited defects of genes in these pathways, lead to several disorders, most of which increase susceptibility to cancer by many fold, and maybe evident by the early age at diagnosis of cancer in these in patients. In addition to genetic alterations, epigenetic modifications such as methylation and histone modification have been shown to bring about genetic instability. In addition, it is likely that the prolonged exposure to

environmental agents and/ or processes may, in concert with individual genetic factors determine the establishment of tumours. Despite these observations, the existence of subsets of tumours that lack an identifiable form of instability has led to skepticism regarding the need for genetic instability in the process of cellular transformation. However, this may indicate that the importance of genetic instability in carcinogenesis differs based on several factors including an individual's genetic background, tissue of interest, baseline mutation rate, environmental exposure, age and time of onset. There also remains the question of whether genetic instability is the driving force behind the process of tumorigenesis or if it is simply a bystander effect of the process. Thus the precise role of genetic instability in the various cancers needs to be defined further. An additional challenge is posed by the prospective identification of cancer stem cells, which call for theory of genetic instability to be reviewed in a new light.

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