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Family Cancer Syndromes: Inherited Deficiencies in Systems for the Maintenance of Genomic Integrity

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Synopsis

Familial cancer syndromes have revealed important fundamental features regarding how all cancers arise through destabilization of the genome, such that somatic evolution can select for the disruption of critical cellular coordinating and regulatory features. We examine those cellular genes and systems whose normal role is to preserve genomic integrity, and relate them to the genetic foundations of heritable cancers. By examining how these cellular systems normally function, we learn how family cancer genes are able to effect the process of tumor progression. In so doing, we additionally gain a clearer picture of how sporadic cancers arise.

Familial Cancers: What can they teach us about cancer in general?

Familial cancers are a heartbreaking tragedy. At the same time they are invaluable research tools for understanding the nature of cancer. We pay homage to these families by investigating their cancers, both to obtain new insights and to learn where we may have previously erred in creating dogmas and paradigms relating to the nature of cancer.

To begin, let us ask how familial cancers relate to current generally accepted viewpoints on the nature and origins of cancer. How can familial cancers arise if tumor progression is a precise set of five or six linear steps as described in the basic Vogelstein pathway [1]? Why would genes be preserved during our evolutionary histories if they were able to generate such mutational and/or epigenetic steps of tumor progression?

Carcinoma genomes are typically extensively altered, in sporadic carcinomas with thousands of genomic events per tumor present in both coding and non-coding sequences. Varying sets of around one hundred mutated genes exist in each tumor cell [2-6]. Especially for sporadic cancers, tumors are genomically heterogeneous between one another, and each tumor is

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genomically heterogeneous at the cellular level, as somatic evolutionary branching generates divergent cellular subpopulations during tumor progression. While targeted therapies directed at systems altered in most of a tumor's cells can shrink the tumor, minority populations of cells lacking the target often lead to disease recurrence. Accordingly, sporadic carcinomas generally have poor outcomes if complete surgical resection is not achieved.

Tumors arise from naturally proliferating cell populations, such as the stem cells and their daughters in the colonic mucosa or esophageal epithelia. In sporadic tumor progression small events are seen first; these are unlikely to damage multiple genes and are unlikely to disable redundant cellular systems [7-9]. With a considerable majority of the genome dedicated to systems for differentiation, development and cell-cell interactions, the probability is low that a small event occurring as an early mutation will kill the cell (Figure 1). Over time, additional mutations occur in that cell lineage, as well as in other similar cells, and most of the cells in such lineages will retain the ability to proliferate. As Nowell anticipated in 1976 [10], some of the cells will have various proliferative advantages, largely arising from greater losses of proliferative controls; over a lengthy course of tumor progression those cells with the most advantageous mutations will tend to predominate. Additional genes further involved in the preservation of genomic integrity often eventually become mutated; if such genes also generate small events, progression will continue to be slow. But if these later genes cause large events, simultaneously impacting many genes at once (e.g. chromosomal deletions), the likelihood becomes high for removing complementing alleles of mutated genes, or redundant genes preserving pathways involving previously mutated genes. Large events early in progression would create a level of damage likely to disable progression, but late in progression a single large event can produce major or even catastrophic changes as earlier small events, and their corresponding mutated genes, suddenly have no backups. The tumor typically presents with numerous small alterations, along with varying numbers of large alterations that can have major impacts on the aggressive nature of the tumor. Genomic diversity in a large population of somatically evolved cells that have lost many of the normal functions guiding organismic cellular behavior combines to create the potentially deadly situation known as cancer.

Familial cancer syndromes represent cases where afflicted members inherit a defective copy of one of the many different genes needed to maintain genomic integrity [11,12]. In a normally proliferating cell of such an afflicted member, once the corresponding nondefective allele is lost by intragenic mutation or deletion, or functionally reduced by amplification of the inherited defective allele, the process of genomic destabilization commences. Daughter cells accumulate genomic damage at a higher rate than that which occurs with environmental damage alone; over time this frequently leads to cancer. Thus, what distinguishes familial cancer from sporadic cancer is that, in familial cancer, only one additional event is required to generate the genomic destabilization facilitating progression. In normal individuals two separate events are required, in order to eliminate the functionality of each allele inherited from the mother and the father. For this reason, the probability of cancer developing in a normal individual is considerably lower than in an individual inheriting a family cancer gene.

Family cancer genes may be directly or indirectly associated with the maintenance of genomic integrity. Contributing to genomic integrity are diverse processes such as the correction of errors in the replication of repeat sequences, processing of DNA flaps inherent in the replication process, activation of checkpoint systems necessary to arrest the cell cycle until DNA damage is repaired, removal of defective DNA bases, and initiation of programmed cell death if genomic damage cannot be efficiently repaired. Familial cancers progress along routes initially or completely driven by the specific genomic integrity maintenance defect; this in turn selects for later routes amenable to the defect [Figure 2]. For example hereditary nonpolyposis colorectal cancer arises from an impaired ability to repair DNA base mismatches; since those few genes containing DNA repeat sequences are far more likely to suffer from such repeat

replication errors, specific genes such as TGF β RII become selected early in tumor progression [13]. Such selection for specific events during progression can result in a tumor that is more genomically homogeneous than occurs with sporadic cancers; in such cases, superior clinical outcomes are generally seen.

Let us now consider the forms of damage to which the genome is susceptible, and the cellular systems that repair or ameliorate each type of damage.

Types of DNA damage [14-16]

1. Base loss

The glycosyl bond linking DNA bases to the sugar-phosphate DNA backbone is labile under physiological conditions. When a base is lost due to cleavage of the glycosyl bond, an abasic site is left behind.

2. Base Modifications

Chemical modifications of bases by deamination, oxidation, alkylation and other processes can all occur spontaneously under physiological conditions. Many environmental carcinogens act through this mechanism.

3. Photodamage

The UV rays from sunlight are capable of inducing a variety of types of damage in DNA. Of these, the most common is the formation of covalent bonds between adjacent pyrimidines in one strand of the DNA.

4. Replication errors

In spite of the high accuracy of the replication systems a number of alterations generating mismatches, small deletions or insertions commonly occur. Most of these alterations are repaired by polymerase-associated proof reading activity and replication-coupled repair.

5. Inter-strand and DNA-protein cross links

Bifunctional alkylating agents, UV rays and ionizing radiation are capable of cross-linking both DNA strands, and DNA and protein molecules.

6. Strand breaks

Ionizing radiation can generate single strand and double strand breaks. This can result in severe chromosomal instability.

DNA Repair Mechanisms

The plethora of damages inflicted on the DNA has evolutionarily given rise to a number of repair mechanisms. They form an intricate system that is specific to the nature of damage and can be broadly categorized as the direct reversal of damage, excision of the damaged region and its precise replacement, or double strand break repair and damage bypass.

1. Direct reversal of damage

In general the direct reversal of damage is not feasible for a cell. In some cases this may occur, such as the photoreversal of cyclobutane pyrimidine dimers (CPD) by CPD photolyase in bacteria, fungi, plants and many vertebrates, but not in mammals. For example, O⁶-methylguanine- DNA methyltransferase is a unique molecule that is permanently inactivated on accepting the methyl group from O⁶-methylguanine to a cysteine on the protein [17,18].

A second type is the reversal of 3-methyl cytosine and 1-methyl adenine to the original base by AlkB (an alpha-keto-glutarate- dependent and iron- dependent oxygenase) [19].

2. Excision repair

Three major highly conserved and partly overlapping repair mechanisms that involve the excision of the damaged base or a region encompassing the damage include mismatch repair, base-excision repair and nucleotide excision repair. These pathways function in the removal of distinct damage utilizing specific proteins and enzymes.

• **Mismatch repair [20,21]**—The components of mismatch repair are MutS, MutL and MutH and uvrD in *E coli*; eukaryotes possess multiple homologs to MutS: MSH2/3/4/5/6 in humans and MutL: MLH1/3, PMS1/2; eukaryotes lack MutH and uvrD homologs. MutS and its human homologs recognize a mismatch and insertions or deletions of up to four nucleotides and binds to them. MutL (and its human homologs) then bind this complex to stabilize it. In *E coli* the hemimethylated state present due to semi-conservative replication is utilized by MutH to recognize the old DNA to serve as the template. The opposite strand is nicked, uvrD (Helicase II) unwinds from the nick and along with an appropriate exonuclease (Exonuclease 1 and FEN1 in humans) the damaged DNA is excised. This is followed by resynthesis using polymerase III (in eukaryotes standard replication proteins are required, viz. PCNA, RPA, RFC, and DNA Polymerase delta) and ligation by DNA ligase. Hereditary nonpolyposis colorectal cancer HNPCC arises from defective mismatch repair genes, particularly MSH2 and MLH1.

• **Base excision repair**—Abasic sites and incorrect bases incorporated as a result of ROS, methylation or deamination for which an N-glycosylase is present are repaired by base excision pathway. The basic steps involved include removal of the incorrect base by an appropriate DNA N-glycosylase to generate an AP site - apurinic or apyrimidinic site (also known as abasic site). AP endonuclease then nicks the damaged DNA to generate a 3'-OH terminus upstream of the AP site. DNA polymerase extends from the 3'-OH available, followed by the excision of the AP site. A spectrum of glycosylases exists that specialize in the hydrolysis of the N-glycosylic bond between the specific base and deoxyribose [22-24]. The glycosylase gently pinches the DNA such that kinks generated by damage ultimately result in flipping out of the base into the enzyme cavity to cleave the base and generate an AP site [24]. AP endonuclease then cleaves the phosphate backbone upstream of the AP site creating a 3'-OH at which DNA polymerase beta fills the single nucleotide gap and removes the baseless sugar residue with its inherent deoxyribose phosphatase activity, if the glycosylase acting earlier lacked a lyase activity [25,26]. XRCC1-ligase3 seals the nick to repair damage by the predominantly used short patch pathway (single nucleotide replaced). An alternative partially utilized long-patch base excision repair functions to repair single strand breaks generated by X-rays. XRCC1, poly (ADP-ribose) polymerase 1 (PARP) and polynucleotide kinase (PNK) protect and trim the single strand ends for repair synthesis [27-30]. DNA synthesis depends on the regular replication machinery of DNA polymerase beta, delta and epsilon and PCNA to replace 2-10 bases, the displaced strand is removed by FEN1 and DNA ligase seals the final nick.

• **Nucleotide excision repair (NER)**—A number of proteins in concert form the nucleotide excision repairosome that recognizes DNA damage based on abnormal DNA structure as well as abnormal chemistry. The repairosome generates a bimodal incision on the flanking regions of the damage site and DNA polymerase fills the resulting gap of about thirty nucleotides [31,32]. This repair system is active in global genome repair and transcription coupled repair and is not specific in damage recognition. Global genome repair is initiated by XRCC-hHR23B that recognizes damage based on chemical alterations to the DNA and structural distortion, and augments the distortion to attract other components [33]. Essentially TFIIH is a general transcription factor whose sub-units XPB and XPD function as helicases to open the DNA

structure around the damaged site [34]. Single strand binding protein RPA stabilizes the structure. XPA is essential for the unwinding of DNA but its exact role in NER is unknown. It may function in the confirmation of damage and the recruitment of proteins to their correct positions with respect to the damage. In the case of transcription-coupled repair CSA and CSB associate with the stalled RNA polymerase to make the damage accessible to TFIIH, XPA and RPA. They function similarly to global genome NER and unwind approximately thirty bases around the damaged site [35]. XPG and XPF/ERCC1 are structure-specific endonucleases that cut at the junction of single and double strand DNA, 3' and 5' to the site on the damage strand respectively [36,37]. The resulting gap is filled by DNA polymerase epsilon or delta assisted by PCNA. And the final nicks are sealed by DNA ligaseI [38-40].

3. Double-strand break repair

Double strand breaks can be repaired accurately by homologous recombination and by non-homologous end joining; the latter is error prone and a less preferred pathway [41-42].

• **Homologous recombination**—The presence of double strand breaks induces activation of cell cycle checkpoints ATM, ATR and p53, discussed in detail later. This results in halting of the cell cycle and recruitment of repair factors. The double strand breaks are repaired in a process similar to homologous recombination that occurs naturally during meiosis [43]. In S phase cells homologous recombination is the preferred choice of repair. RAD50/MRE11/NBS1 exposes the 3' end of the breaks via its 5'-3' exonuclease activity and along with RPA coats the exposed ends to form a nucleoprotein filament. Various other RAD51 proteins involved include RAD51B, C, D, RAD52, XRCC2 and XRCC3. The RAD51 complex along with RAD54 (SWI/SNF DNA dependent ATPase) induces exchange between single strand DNA and its homologous sequence. Strand invasion is followed by conservative replication as both strands are newly synthesized. The Holliday junctions formed are resolved by resolvase and nicks are ligated by DNA ligase. In some cases the 3' resected ends expose complementary regions of repeat sequences and they are ligated with the loss of a few nucleotides resulting in a small deletion. In non-dividing cells, finding the homologous chromosome is not feasible and non-homologous end joining repairs double strand breaks. The familial breast and ovarian cancer genes BRCA1 and BRCA2 encode proteins associated with the RAD51 repair complex.

• **Non-homologous end joining [44]**—The Ku heterodimer (Ku70 and Ku80) is a DNA binding ATPase and possibly a helicase. It binds to DNA ends, stem loops and bubble structures as well as single- and double stranded DNA. Along with DNA-PK_{CS} (DNA dependent protein kinase catalytic sub-unit) encoded by XRCC4 it forms a trimeric protein; DNA PK_{CS} phosphorylates the Ku proteins to activate their ATPase activity. The broken DNA strands in close affinity to one another allows XRCC4 to bind as a dimer to the DNA ends. Each monomer in turn binds a DNA ligase IV. Prior to ligation of the ends any flaps present are removed and the gaps are filled potentially by Mre11 proteins. This repair may involve the loss or gain of a few nucleotides, a price worth paying versus the damage double strand breaks in DNA would generate.

4, Damage bypass/tolerance

This mechanism has evolved to avoid stalling of replication forks for too long, which leads to cell death. So far up to seven polymerases that function in by-pass synthesis have been identified by various laboratories [45,46]. These include Pol ζ, Pol η, Pol δ, Pol ι, Pol κ, and REV1. Recombinational damage bypass utilizes the nascent strand as a template to by pass the damage site. Translesion bypass synthesis utilizes Pol η and Pol ι, they bind behind the stalled replication machinery to cause a conformational change. This orients Pol η to synthesize across the damage lesion. Pol ι completes the process by incorporating a few more nucleotides and allows the replication machinery to continue forth [47,48].

Overview of the Cell Cycle

Major proteins involved in cell cycle regulation are protein kinases consisting of a regulatory subunit called cyclin and a catalytic subunit called cyclin dependent kinase (CDKs). Cyclins as the name suggests demonstrate a cyclic pattern of expression throughout the cell cycle. The cyclins D, E, A and B peak at G1, G1-S, S and S-G2, and G2-M respectively. The various CDKs associate with different cyclins at each phase. The CDKs are regulated by CDK inhibitors - INK (inhibitors of CDK4) and CIP/KIP proteins. INK family proteins include p15^{INK4b}, p16^{INK4a}, p18^{INK4c} and p19^{INK4d} [49-52]; these inhibit the catalytic subunits of CDK4/6, while CIP/KIPs include p21^{Cip1}, p27^{Kip1}, p57^{Kip2} and bind cyclin D (positively), E and A (negatively) [53-55]. The Cdk proteins are phosphorylated by Wee1/Myt1 kinases and are inactive, dephosphorylation by CDC25 phosphatases (CDC25A, B and C) activates the Cyclin-Cdk complex [56]. The CDC25 phosphatases are important in cell cycle progression, and also involved in checkpoint induced arrest. CDKN2A gene defects underlie some cases of familial melanoma.

The retinoblastoma (Rb) family of proteins is a pocket protein that binds and inhibits the E2F family of transcription factors when hypophosphorylated [57]. E2F functions in the transcription of genes required for cell cycle progression. The Rb family includes Rb, p107 and p130; and is also capable of inducing chromatin remodeling via HDACs. Rb in mutated form is the gene underlying familial retinoblastoma.

At G0-G1

Cyclin D is induced by the Ras-Raf-Mek1-Mek2-ERK cascade in the presence of growth factors and associates with Cdk4/6 and CIP. Cyclin D competes with INK4 proteins that inhibit Cdk4/6. Cyclin D-CDK4/6-CIP enters the nucleus and CDK4/6 are phosphorylated at threonine residues by CDK activating kinases. The phosphorylated cyclinD-CDK4/6-CIP complex is active and can phosphorylate its down stream target - most importantly Rb. Cyclin D turn-over involves the Ras-PI3K and AKT signaling ultimately resulting in its ubiquitin-dependent proteosomal degradation.

At mid-G1

Rb binds E2F a major transcription factor involved in the transcription of genes required for DNA replication, deoxyribonucleotide synthesis and various cyclins and CDKs [58]. Rb bound to E2F blocks its transactivation domain and recruits HDAC1 (histone deacetylase 1) resulting in a compressed and closed chromatin that represses promoters. However the phosphorylation of Rb by CyclinD-CDK4/6 eases the repression on E2F and allows for cyclin E synthesis. The sequestering of Cip/Kip proteins to Cyclin D lowers their inhibitory action on Cyclin E-CDK2.

In late-G1

Increased Cyclin E binds to CDK2 and hyperphosphorylates Rb to disrupt its association with E2F. E2F is now capable of transcribing the required S-phase genes along with transcription factors E2F 1-3. Cyclin E- CDK2 phosphorylates Rb and Cip inhibitor p27^{Kip1} resulting in its degradation and histone H1 required for chromatin remodeling during replication. Cyclin E-CDK2 induces the transition to S phase. The cells have passed a restriction point and are now committed to undergo one round of replication.

At S phase

E2F 4/5 present in the nucleus in G1 moves into the cytoplasm while E2F 1-3 transcribes S phase genes. Cyclin A-CDK2 maintains Rb in a hyperphosphorylated state and persists throughout S phase. During late S phase, Cyclin A-CDK2 phosphorylates E2F 1-3 along with

Rb. This results in dissociation of the transcription factors from the promoter, a step that precedes transition to G2. As cells transit through S phase Cyclin E is degraded.

G2 phase

During early-mid G2 CDK1 accumulates and is phosphorylated by Wee1 and together with Cyclin B forms an inactive complex. At late G2 Cdc25 dephosphorylates Cyclin B-CDK1; the active form phosphorylates mitotic substrates. Cyclin A is degraded during G2. At G2/M Protein phosphatase1 (PP1) dephosphorylates Rb. Hypophosphorylated Rb complexes with E2F and recruits HDACs to prevent transcription of S phase genes. The cells then proceed to mitosis to divide and form two daughter cells [59,60].

Cell Cycle Checkpoints

Checkpoints function to ensure the completion of a step prior to proceeding to the next phase of the cell cycle. In essence, they monitor the accuracy and fidelity of DNA replication and repair. The various proteins involved can broadly be classified into sensors, transducers and effectors. Sensor proteins recognize damaged or abnormal DNA and set off a cascade of reactions through the transducers to finally stall the cell cycle. Transducers are often protein kinases that convey and magnify the damage signal received from sensor proteins. Effector proteins are generally phosphorylated by the transducer proteins and are actively involved in preventing progression to the next phase of the cell cycle. Checkpoints occur during various phases of the cell cycle. At G1 when a cell detects DNA damage, it stalls progression to S-phase until the damage is repaired, so as to prevent the replication of damaged DNA. During S phase when replication forks stall due to encounter with damaged DNA or lack of dNTPs the cell prevents the firing of new origins and stalls at S phase to prevent entry to M phase. Cells enter M phase after the DNA has undergone one complete accurate round of duplication. Spindle checkpoints monitor the precise and faithful segregation of chromosomes into the daughter cells. A variety of DNA binding proteins act as sensors that detect the presence of damage, and are often involved in repair and/or replication [61]. Important proteins involved in checkpoints include tumor suppressors such as p53 and Rb, ATM (*ataxia telangiectasia mutated*) and ATR (*ATM and RAD3--related*) that are part of the phosphatidylinositol-3 kinase-like kinase (PIKK) family and effectors such as Chk1 and Chk2.

Sensors in checkpoints

RFC1 (Replication factor C 1)-like protein Rad17 is involved in the detection of damage and further recruits PCNA (proliferating cell nuclear antigen)-like proteins, the Rad9, Rad1 and Hus1 (9-1-1) complex to the damaged DNA sites. The 9-1-1 complex functions as a sensor and facilitates the phosphorylation and activation of ATR and ATM [62,63].

ATM is primarily activated in response to double strand breaks (induced by ionizing radiation) and DNA damage, while ATR plays a critical role in stalling the replication fork in response to UV damage or other stress [64,65]. Often DNA damage results in the activation of both ATR and ATM and they appear to phosphorylate similar substrates.

Mediators in checkpoints

Proteins that can be broadly classified to function as mediators included BRCT (BRAC 1 C-terminus repeat domains) - containing proteins involved in protein-phosphoprotein interactions and MRN complex proteins that recognize double strand breaks. The BRCT- containing proteins include p53 binding protein (53BP1), Mediator of DNA damage Checkpoint 1 (MDC1), Topoisomerase binding protein (TopBP1) and BRAC1.

The phosphorylated and activated ATM phosphorylates γ H2AX, the MDC1 protein functions as a bridge between γ H2AX and NBS1 from the MRN (MRE11-Rad50-NBS1) complex [66-68]. The mediator proteins associate via γ H2AX and form a protein scaffold microscopically visible as foci that targets ATM to the damaged site and facilitates ATM signaling [69].

Transducers in checkpoints

ATM, ATR, Chk1 and Chk2 are the vital signal transducers involved in cell cycle arrest.

ATM is present as a homodimer, with its kinase domain containing a vital activating Serine 1981 residue blocked. Sensing of DNA damage via a change in chromatin structure, or signaling from an upstream sensor protein results in the activation and autophosphorylation of ATM and its dissociation to an active monomer [70]. The activated ATM is involved in phosphorylation of mediator proteins (such as NBS1, BRAC1, 53BP1, MDC1, SCM1) and in turn gets recruited to the site of damage [71]. In the absence of mediator proteins such as NBS1 or BRAC1 ATM phosphorylates downstream nucleoplasmic effector proteins such as p53. The various effector proteins are phosphorylated in context to the cell cycle stage [72]. Cells lacking ATM are viable, suggesting it is not essential for normal cell cycle and is primarily involved in double strand break signaling pathways [65]. In humans, inherited ATM mutations causes ataxia telangiectasia, an autosomal recessive disease associated with lymphoid malignancies.

ATR and ATRIP (ATR interacting protein) exist as a heterodimer with functional kinase activity [73,74]. Its cellular functions are controlled by its subcellular localization. ATRIP interacts with RPA (Replication protein A) that binds single stranded DNA. Through this interaction ATR is recruited to the DNA replication forks. Various types of damage or stress to the cell such as UV irradiation, bulky adduct formation, alkylating agents or DNA crosslinks result in stalling of the replication forks. Cells lacking ATR are non-viable as it responds to various types of cellular stress that affect the rate of replication fork progression and also plays a role in monitoring the progression of the replication fork under normal conditions [75]. ATR is capable of slowing down origin firing in response to DNA damage [76]. ATR, the 9-1-1 complex at single stranded DNA and claspin are required for the phosphorylation and activation of CHK1 described below. Claspin is unrelated to mediators involved in the ATM pathway for double-strand breaks but binds chromatin structures created by active replication forks [77].

Chk1 and Chk2. Chk1 serine residues 317/345 and Chk2 threonine residue 68 are phosphorylated by ATR and ATM preferentially to activate their kinase activity [78]. Chk1, similar to ATR is essential for cell viability and its deficiency results in early embryonic death and gross chromosomal abnormalities [79,80]. Chk1 targets CDC25A, CDC25B and CDC25C and down regulates their phosphatase activity whereas Chk2 targets CDC25A for ubiquitin-mediated degradation at the intra-S phase checkpoint [71]. Chk2 functions essentially to mediate apoptosis via p53 [81].

Effectors in checkpoints

p53 and the Cdc25 phosphatases are the important effector molecules that bring about arrest in cell cycle progression.

P53 and the G1 Checkpoint

The tumor suppressor p53 plays an important role in determining the fate of damaged cells, either inducing cell cycle arrest or apoptosis. p53 transcriptional activity is post-translationally controlled. A defective p53 gene is responsible for the wide variety of cancer types seen in patients with the Li-Fraumeni familial cancer syndrome. Mdm2 is a ubiquitin ligase that

regulates the p53 levels in a cell via ubiquitylation, nuclear export and proteasome degradation [82]. ATM and ATR phosphorylate p53 at Serine 15 along with other sites, this inhibits its interaction with Mdm2 [83]. ATM also phosphorylates Mdm2, reducing the nuclear export and degradation of p53. This leads to p53 stabilization. The stabilization and accumulation of p53 results in increased transcription of targets such as p21^{CIP1/WAF1} that inhibits cyclinE/Cdk2 activity and results in G1 arrest [53,84]. This suppresses the Rb/E2F pathway, and results in a sustained G1 arrest. The degradation of Cyclin D also brings about G1 arrest [60,86].

ATR and G1/S Checkpoint

During late G1 the quantity of ATR and Chk1 increase along with amounts of CDC25A. Under normal circumstances ATR/Chk1 maintains the levels of CDC25A via its phosphorylation and ubiquitin mediated proteasomal degradation [87]. CDC25A activates the Cyclin E/Cdk2 complex via the dephosphorylation of Cdk2. In response to damage ATR/Chk1 and Chk2 downregulates CDC25A resulting in Cyclin E/Cdk2 inactivity and cells arrest at G1/S [88].

S Checkpoint

Similar to ATR mediated G1/S arrest; at S phase Cyclin A/Cdk2 and Cyclin E/Cdk2 are inhibited preventing the completion of DNA synthesis. The inhibition of CDK2 blocks the loading of CDC45 onto chromatin, required for recruitment of DNA polymerase α , thus inhibiting origin firing. A parallel pathway involving the BASC (BRCA1-associated genome surveillance complex) that includes ATM, NBS1, BRCA1 has been discovered by the attenuation of S phase checkpoint activation, but remains to be characterized completely [89-91].

G2/M Checkpoint

CyclinB/Cdk1 mediates the transition from G2 to M via CDC25C activation. CDC25C dephosphorylates Cdk1 leading to its activation and progression to M phase. DNA damage and the resulting activation of ATR/Chk1 and ATM/Chk2 targets CDC25C for inactivating phosphorylation. CDC25C is sequestered in the cytoplasm preventing the dephosphorylation of Cdk1 thus inhibiting G2/M progression [92].

Spindle Checkpoints

Successful mitosis involves the condensation of chromosomes, formation of spindle fibers, the alignment and separation of sister chromatids to both poles, followed by cytokinesis to generate daughter cells [93,94]. The various proteins that play a role in spindle checkpoint and are known to be deregulated or mutated in cancers include Mad proteins (*mitotic arrest deficient*), Bub proteins (*budding uninhibited by benomyl*), and at the centrosome - AuroraA [95,96]. The spindle checkpoint monitors the correct alignment of chromosomes at the equatorial plane during metaphase and their attachment to spindle fibers to ensure accurate chromosomal segregation. In a study by Sotillo et al, mice that over express Mad2 showed enhanced tumor formation in more than 50% of those analyzed, and these tumors were characterized by numerous chromosomal rearrangements [97]. The Mad proteins are involved in the separation of sister chromatids and overexpression results in tetraploidy.

Cyclin B-Cdk1 is also known as the mitosis-promoting factor (MPF). CyclinB-Cdk1 phosphorylates condensins, the proteins involved in condensing interphase DNA [98]. Sister chromatids are held together at the centomere and along the chromosome arms through the protein cohesin (Smc proteins). Each chromosome attaches to the microtubules emanating from either pole via its kinetochore. It has been suggested that a checkpoint may exist that monitors

the attachment of chromosomes at the kinetochore and may function via monitoring the tension generated by the chromosome- kinetochore interaction [99,100].

An essential checkpoint exists in the form of the Anaphase promoting complex (APC). The APC is a complex of ubiquitin conjugating (E2) and ligating (E3) enzymes [101]. Mad1/2 complex present at unoccupied kinetochores, form a mitotic checkpoint complex (MCC) consisting of Mad2, BubR1, Bub3 and cdc20. The MCC binds and inhibits APC activity. APC-cdc20 is activated when the spindle checkpoint is satisfied and is also dependent on MPF (maturation promoting factor - CyclinB/Cdk1), which is involved in the break down of nuclear membrane and formation of spindle fibers at the start of metaphase [102]. The cohesion complexes are cleaved by Separase (Esp1) to separate the sister chromatids [103]. This allows for the relocation of the chromosomes from the metaphase plane to the two poles of the newly forming daughter cells. All along separase is inactive via its interaction with securin (Pds1); APC destroys securin to activate separase [104]. APC also degrades CyclinB, which leads to decrease in MPF a requirement to exit mitosis.

DNA Repair, Cell Cycle Checkpoints and Chromosomal Segregation Defects in Familial Cancer Syndromes

Defects in excision repair and double strand break repair pathways predispose cells to form cancers, as a result of increased genomic instability [105]. Defects in mismatch repair genes MLH1 and MSH2 are involved in the generation of microsatellite instability observed chiefly in (HNPCC) Hereditary Non-Polyposis Colon Cancer [106,107].

Defects in nucleotide excision repair are associated with various UV sensitive syndromes. They include xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD) [108,109]. XP patients show elevated rates of skin cancers, while CS and TTD demonstrate premature aging but no predisposition to cancer. This is because the complementation groups of genes involved in CS and TTD are major players in transcription coupled NER and not global genome NER, like those of XP. Defective or stalled transcription results in activation of p53 cascade resulting in senescence or apoptosis. However NER defects that allow the accumulation of mutations, as in the case of global genome-NER, are prone to cancer.

Inherited defects in repair allow an increase in mutational burden and an increase in genomic instability, which may occur at regions of a few nucleotides or over much larger regions. These individual defects in repair genes tend to result in corresponding types of mutations and corresponding sets of mutated genes. As described above, there are numerous systems, and even more genes, involved in the maintenance of genomic integrity. Defects in only a few of these are currently established to represent true family cancer genes. As to the rest of the above genes, complete loss of function in most cases would inevitably be lethal. However more subtle reductions in functionality for these genes (as well as possibly other classes of genes) would be expected in many cases to impact the probability of the development of malignancies, or the rate of progression as affecting the age of onset of disease. Discerning cases with modest contributions will be difficult among the statistical noise of cancer incidence; large scale comparisons with single nucleotide polymorphisms (SNPs) can be expected to reveal some of these weaker associations.

The well known family cancer genes have very substantial biological consequences. As described in the following chapters, such consequences in turn have significant impact in the clinic.

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What difference does size of events make?

$$\text{Total genomic damage} = (\text{size of events}) \times (\text{events per mitosis}) \\ \times (\text{number of mitoses})$$

With regard to total genomic damage

$$(\text{Large events}) \times (\text{short time}) = (\text{small events}) \times (\text{long time})$$

for a diploid cell:

$$\text{Probability of a lethal event} \propto (\text{size of event})^2$$

Figure 1.

Relatively small genomic events occurring over a lengthy period of time, with ongoing selection for cellular viability, is more likely to generate greater overall genomic alteration, while producing extensive genomic heterogeneity within the tumor cell population. In contrast, relatively large events are more likely to destroy both alleles of any given gene, in turn often generating cellular lethality before progression can go far. Large events occurring late in progression can simultaneously reveal many small events arising earlier in progression, pushing progression across the malignancy threshold.

Figure 2.

Sporadic tumor progression, for example as characterized for colorectal carcinoma or anaplastic thyroid cancer, is a diffuse evolutionary process. Certain more advantageous events, starred, will be selected for at a somewhat greater frequency, although the diverse routes taken by different cell lineages within the progressing tumor ultimately combine to produce a genomically heterogeneous tumor. Family cancer genes can lead to specific types of genomic events (**familial-1**), as with hereditary nonpolyposis colorectal cancer arising from defective DNA mismatch repair, leading to specific genes becoming mutated during the course of progression. Other family cancer genes (**familial-2**) that promote a broader spectrum of types of genomic events, as with BRCA1 and BRCA2, can lead to more genomically diverse tumors resembling sporadic cancers.