African Journal of Pharmaceutical Research & Development

Vol. 10 No.2; pp. 060-078 (2018)

COLORECTAL CANCER AND ASPIRIN: A LITERATURE REVIEW

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ABSTRACT

The rise in the prevalence of colorectal cancer (CRC) in Nigeria and the world as a whole is of great concern. Metastatic colorectal cancer (mCRC) in particular is still difficult to treat resulting in poor prognosis. Evidence suggests that the NSAID, aspirin, has the potential to decrease incidence of, or mortality from, a number of cancers through several mechanisms of action, which has led to the recommendation of low dose aspirin for the prevention of CRC by the Unites States Preventative Services Task Force. This literature review looks into the hallmarks of cancer, types and pathways of colorectal cancer with emphasis on the different treatments, clinical trials. It also looks into the different proposed mechanisms of action that aspirin follow to execute its effect on the growth of cancer cells with the hope that it will also be adopted as a standard in the chemoprevention of colorectal cancer in Nigeria. This paper will hopefully serve as a reference point for interested researchers that are especially new to this field.

KEYWORDS: **Aspirin, colorectal cancer, chemotherapy, immunotherapy, metastatic colorectal cancer.**

ABBREVIATIONS

5-fluorouracil (5-FU), adoptive cell transfer (ACT), AMP kinase (AMPK), apoptosis inducing factors (AIF), B-cell lymphoma 2 (BCL-2), B-cell lymphoma extra-large (BCL-XL), BCL-2 associated X protein (BAX), BCL-2 homologous antagonist killer (BAK), BCL-2-like protein 11 (BIM), BCL-2-like protein 2 (BCL-W), BH3 interacting-domain death agonist (BID), BRAF (a human gene that encodes the protein B-Raf), carcinoembryonic antigen (CEA), chromosome instability (CIN), colorectal cancer (CRC), gene,cell cycle rest (G0) phase,metastatic CRC (mCRC), microsatellite instability (MIN), mismatch repair (MMR) gene, mitogen-activated protein-kinase (MAPK), mitotic spindle checkpoint (MSC), nuclear factor-κB (NF-κB), phosphoinositide 3-kinase (PI 3-K), platelet-derived growth factor (PDGF), reactive oxygen and nitrogen species (RONS), retinoblastoma-associated (RB),toll-like receptor (TLR), tumour associated antigen (TAA), tumour growth factor α (TGFα), tumour growth factor α (TGFα), tumour-infiltrating lymphocytes (TIL), vascular endothelial growth factor-A (VEGF-A).

INTRODUCTION

Cancer arises as a consequence of the accumulation of genomic changes, which include genetic mutations, disruption in the functionality of these genes and deregulation of signalling pathways. These genetic mutations result in an increase in functionality of oncogenes and a decrease or loss in the functionality of tumour suppressor genes [1]. These genetic changes result in alterations in cell physiology termed as the hallmarks of cancer [1,2,3].

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DISCUSSION

The Hallmarks of Cancer

The genomic changes were initially thought to be as a result of six alterations in cell physiology termed as the six hallmarks of cancer. They included sustaining proliferative signalling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis and resisting apoptosis [1] (Figure 1). Later on, cancer-related inflammation was included as the seventh hallmark of cancer [2] (Figure 1).

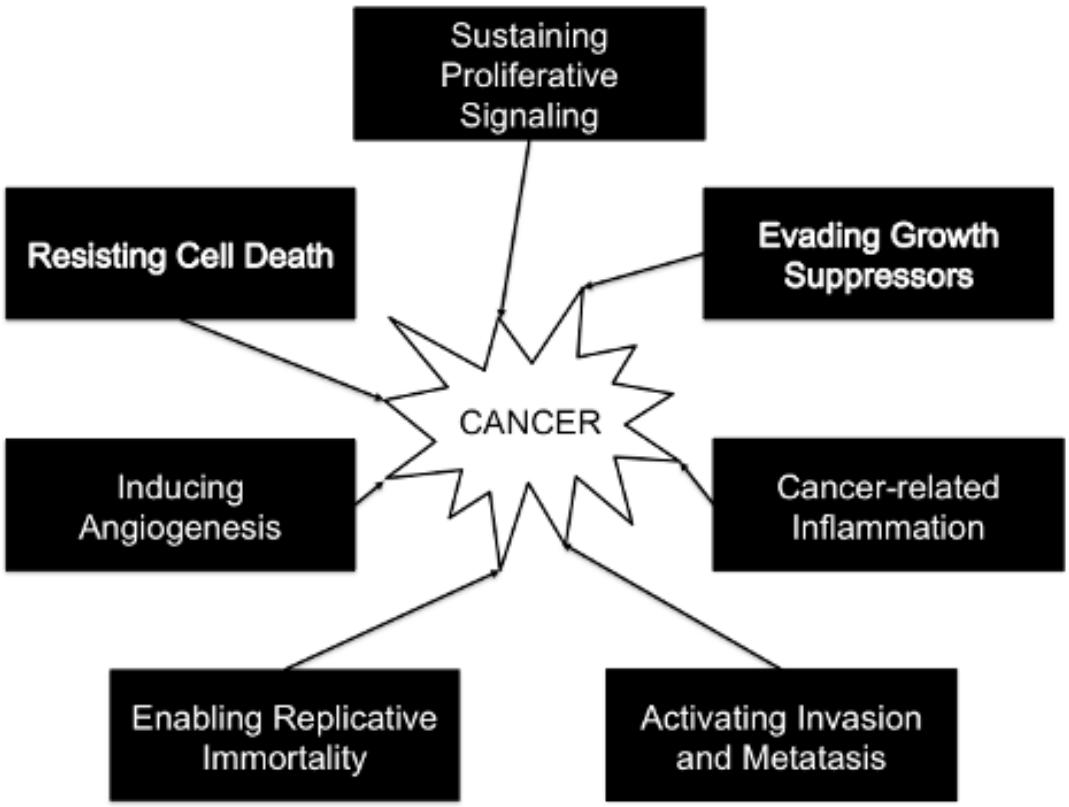

Figure 1: The seven hallmarks of Cancer. [Adapted from (Colotta *et al.*, 2009, Hanahan and Weinberg, 2000, Hanahan and Weinberg, 2011)

Sustaining Growth Signalling

Mitogenic growth signals are required for cells to change from a rest phase to a proliferative phase by transmembrane receptors [4]. Normal growth signals are mimicked by oncogenes [5]. This enables tumour cells to generate their own signalling programme. For example, cancer cells do not rely on normal cells for the production of growth factors such as platelet-derived growth factor (PDGF) and tumour growth factor α (TGFα), but rather produce these growth factors independently. In addition, cancer cells may also send signals to normal cells, which in turn supply the cancer cells with various growth factors [6]. Cell surface receptors responsible for this signalling also undergo deregulation resulting in overexpression. For example, the epidermal growth factor receptor (EGFR) when overexpressed become hyper-responsive to background levels of growth factor [7,8] or ligand-independent signalling [9]. Similarly, somatic mutations in the DNA of cancer cells result in an increase in signalling through the Raf to mitogen-activated protein-kinase (MAPK) pathway [10] and the phosphoinositide 3-kinase (PI 3-K) signalling pathway [11].

Cancer cells can also choose to express extracellular receptors that produce proliferative signals, which could lead to resistance to apoptosis and entry into the active cell cycle. An example is the effects of integrin on growth factors [12].

Evading Growth Suppressors

Antiproliferative signals are responsible for maintaining tissue homeostasis. Antigrowth signals from tumour suppressor genes block cell proliferation either by forcing the cell cycle into rest

(G_0) phase or by causing apoptosis [1,3]. Examples of tumour suppressor genes include *RB* (retinoblastoma-associated) and *TP53*. The *RB* gene, as part of a wider network acts as a 'gatekeeper' to cells for entry or progression into the cell cycle [13] and its inactivation can lead to cellular proliferation. *TP53* gene regulates cell growth or division by halting cell cycle progression or activating apoptosis when stress signals, as a result of damage to the genome, are received [3]. However, its inactivation also leads to cell growth.

Another mechanism in which cancer cells operate to evade growth suppressors is by corrupting the TGF-β pathway [14], where TGF-β regulates the G_1 phase of the cell cycle by suppressing *c-myc* gene and also causes synthesis of p21 and p15 proteins, which inhibits G_1 cyclin CDK leading to cell apoptosis [15].

'Resisting Cell Death'

Resisting apoptosis, also known as programmed cell death, is a contributing factor in cancer development [16,17,18]. Apoptosis is a step-by-step process that takes place within 2 h. It involves disruption of the cellular membrane; break down of the cytoplasmic and nuclear organelles, degradation of the chromosomes, and fragmentation of the nucleus. Finally, the shrivelled cell is engulfed by nearby cells by a process known as phagocytosis [19]. The mitochondria release cytochrome C, a catalyst for apoptosis when proapoptotic signals are received [20]. Proapoptotic proteins include BCL-2 associated X protein (BAX), BCL-2 homologous antagonist killer (BAK), BH3 interacting-domain death agonist (BID) and BCL-2-like protein 11 (BIM) while the antiapoptotic proteins include B-cell lymphoma 2 (BCL-2), B-cell lymphoma-extra large (BCL-XL) and BCL-2-like protein 2 (BCL-W), all of which are of the BCL-2 family [21]. Upon DNA damage, p53 tumour suppressor upregulates BAX, enabled by VDAC2 (voltage-dependent anion channel 2), a channel responsible for the transport of low molecular weight metabolites, which results in the release of cytochrome C by the mitochondria [1,22].

Autophagy, another form of cell death, is a degradation system that enables the delivery of cytoplasmic materials of aged cells during starvation conditions to lysosomes and is important in maintaining cell homeostasis [23,24]. Insufficiency of survival signals leads to the downregulation of the PI 3-K signalling pathway, which results in the stimulation of autophagy and/or apoptosis [25].

Enabling Replicative Immortality

Telomeres, which are composed of several thousand repeats of a short 6-base-pair sequence element, protect the ends of chromosomes and are mainly responsible for proliferation in cells [26]. The loss of a number of telomeric DNA base-pair from the ends of every chromosome occurs after every cell cycle. Progressive reduction in number of the telomeres results in their inability to protect the ends of chromosomal DNA, triggering cell senescence and eventually death. In other words, end-to-end chromosomal fusion occurs due to the chromosome being unprotected, which eventually leads to cell death [27]. Telomerase, an enzyme that adds telomeres to the ends of chromosomes permits indefinite replication and is almost absent in nonimmortalized cells but expressed in high levels in immortalized/cancer cells [3]. This means that cells are resistant to apoptosis and replicate continuously.

Inducing Angiogenesis

Angiogenesis, the growth of new blood vessels is carefully regulated. It begins with the birth of new endothelial cells and their assembly into tubes (vasculogenesis) [28] and then the sprouting of new vessels from existing ones (angiogenesis) [1,29]. This happens to be switched on and off in processes such as wound healing and female reproductive cycling. However, in tumour progression, the switch remains turned on causing continuous sprouting of blood vessels that feed cancer cells [30]. An inducer of angiogenesis is the vascular endothelial growth factor-A (VEGF-A), which can be upregulated by hypoxia and signalling by oncogenes [31].

Activating Invasion and Metastasis

Metastasis, the distant travel or spread of tumour cells, is the cause of about 90% of mortality from cancer [32]. The shape and ability of cells to attach to other cells is altered in cancer cells due the loss of E-cadherin, a cell-to-cell adhesion molecule. The loss of E-cadherin enables cancer cells to move away from assembled epithelial cell sheets and move freely thereby losing its state of quiescence [33,34]. N-cadherin, an adhesion molecule expressed in migrating neurons is upregulated in invasive cancer cells [35,36].

Cancer-related Inflammation

As far back as the 17th century, the relationship between cancer and inflammation was hypothesised by Rudolf Virchow [37]. This hypothesis has since then been reiterated by other scientists [2,3] and can easily be proven due to the development of better markers for histochemical staining that can accurately identify distinct cell types of the immune system [38].
Inflammation, a simple but powerful defence mechanism programmed to halt tissue damage and stimulate repair [39], leads to the progression of the other six hallmarks of cancer by activating growth factors, which sustain proliferative signalling, survival factors that resist cell death, factors that induce angiogenesis, stimulating genetic mutation [3,40] due to increased levels of nuclear factor-κB (NF-κB), reactive oxygen and nitrogen species (RONS), cytokines, prostaglandins and microRNAs [41].
Ten hallmarks of cancer with corresponding therapeutic targets were subsequently described in 2011 [3] and include;

Genome Instability and Mutation

Mutations found in certain genes of cancer cells lead to their dominance in population [3]. For example, inactivation of tumour suppressor genes due to *TP53* mutations [42,43], inactivation of MMR genes due to mutations [44], chromosome instability (CIN) and microsatellite instability (MIN) [45].

Deregulating Cellular Energies

In normal cells, under anaerobic conditions, glycolysis is the main process for energy metabolism with a small amount of pyruvate delivered to the mitochondria [3]. However, cancer cells are able to redirect their energy metabolism even under aerobic conditions to glycolysis. This process known as 'aerobic condition' is accompanied by low adenosine triphosphate (ATP) production and often compensated for by an increase in uptake and utilization of glucose [46]. Two different cell populations can be found in some cancer cells with different pathways for energy metabolism. One of the populations are glucose-dependent cells that produce lactate (Warburg-effect) while the other uses the lactate produced by neighbouring cells as their source of energy [47]. This indicates that normoxia or hypoxia is not a permanent condition for survival in cancer cells but this fluctuates depending on the condition the cells find themselves [48].

Avoiding Immune Destruction

Hanahan and Weinberg (2011) suggested this hallmark with some considerations that it was not a firmly established contributing factor. However, the emergence of immunotherapy in the treatment of cancer reiterates failure of the immune system to detect and destroy cancer cells as one of the hallmarks of cancer [49,50, 51]. For example, there is better prognosis in colon and ovarian tumour patients with high levels of killer lymphocytes as compared to those that lack such cells as part of their immune system [38]. It has also been found that cancer cells are able to secrete immunosuppressive factors that disable T lymphocytes/killer cells [51] and thus the emergence of checkpoint inhibitors such as ipilimumab, tremelimumab and pembrolizumab used in the treatment of some cancers [52,53,54].

Colorectal Cancer

Colorectal cancer (CRC), which includes cancer of the large bowel and rectum, is the third most common cancer in men with over 750,000 cases and the second in women [55] with about 600,000 cases worldwide [56]. While this is becoming a global phenomenon, over 50% of these cases are found in more developed regions of the world, the estimated incidence rates being higher in Australia/New Zealand and the lowest in West Africa. However, there is a poorer survival rate in the less developed regions of the world such as West Africa. In the UK, over 40,000 people were diagnosed with this disease in 2014 alone with almost 16,000 deaths that same year, which translates to roughly over forty deaths per day [57]. Unfortunately, incidence rates of CRC in the UK have increased by 6% over the last ten years. In Nigeria, colorectal cancer was the tenth most prevalent type of cancer, however in the late 90s, it had moved to the fourth position [58]. In the year 2007, records from University of Ibadan Teaching Hospital cancer registry, indicated approximately 70 cases per year for four years [59]. In Jos, Plateau state, records show about 16 cases of colorectal cancer annually [60] and just over 12 cases per year in the northern city of Kano [61]. The median survival duration of patients with metastatic CRC is about six months if untreated [62].

Over 90% of colorectal cancers are adenocarcinomas and about 1% of these are lymphomas [63].
CRC arises as a consequence of the accumulation of variable and specific changes to the genome and these genomic changes alter normal cell function [64]. Progression of CRC to metastasis from normal cells is as a result of the accumulation of abnormalities in the signalling pathways. Most CRC harbour *APC* mutations [65], which usually occur in the early stages of carcinogenesis leading to the deregulation of the Wnt signalling pathway. Some tumours harbour *TP53* mutations that lead to loss of activity while others bear mutations of the *KRAS* oncogene, which leads to deregulation of the EGFR signalling pathway or the up-regulation of β-catenin resulting in deregulation of the Wnt signalling pathway, ECM (extracellular matrix) and EMT (epithelial-mesenchymal transition) [64] (Figure 2).

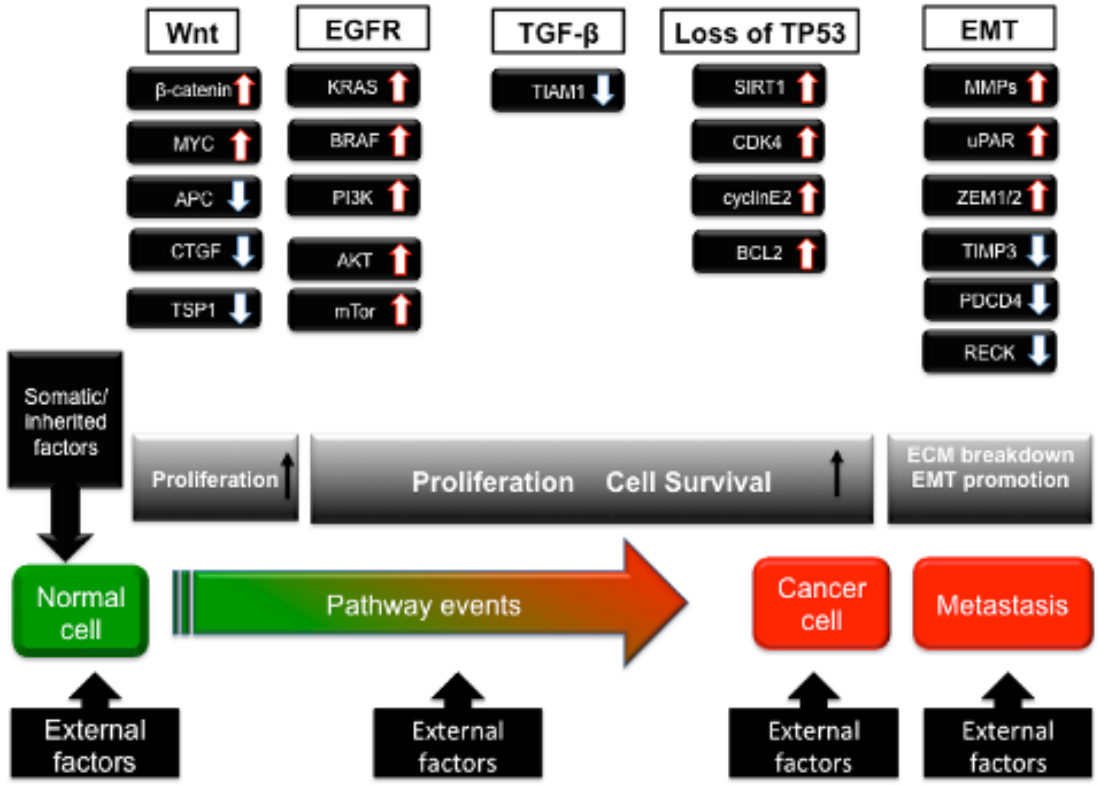

Figure 2: CRC tumourigenesis. [Adapted from (Bustin and Murphy, 2013)].

Types of Colorectal cancer (CRC)

Based on clinical and pathological data, CRC can be divided into three main types [66]; namely:

Adenocarcinomas

An adenocarcinoma can be described as a lesion in the colon or rectum containing clear abnormality in tissue growth (epithelial neoplasia) [67]. Adenocarcinomas develop from the adenoma and are the most common type of CRC. They are found mostly in the left hand side of the large intestine.

Replication errors (RER) Colorectal cancer

Replication errors (RER) CRC have tumours with widespread RERs. They make up about 90% of CRC in patients with hereditary non-polyposis colorectal cancer (HNPCC) syndrome [68,69]. They are mostly found on the right hand side of the bowel [70].

Ulcerative colitis-associated Colorectal cancer (UCACRC)

Ulcerative colitis-associated (UCA) CRC are found mostly on the left hand side of the bowel and usually develop from a distant dysplasia, which can be found in the rectum or sigmoid colon [71]. Mutations at APC and KRAS loci occur less frequently in UCACRC when compared with the other types of CRC [66].

Pathways involved in the development of CRC

Cancer is as a result of the accumulation of gene mutations that eventually lead to uncontrolled cell proliferation [72]. Sources of which include hereditary [(H) germline mutations], environmental factors [(E) such as exposure to UV, cigarette smoke] or due to Replicative mutations (R) [73]. Approximately three mutations occur whenever a normal human stem cell divides [74] and the number of these normal cell divisions determines cancer risk in most mouse model organs [75].

Genetic instability drives cell mutations, which result in the development of different cancers [76]. Genetic alterations in tumours can be divided into four main categories; namely; (i) Subtle sequence changes comprising of a few base pair substitutions, deletions or insertion of nucleotides, (ii) Alterations in chromosome number, which involves losses or gains of whole chromosomes, (iii) Chromosome translocations that results in fusions between two different genes, and (iv) Gene amplifications that results in the formation of 'amplicons', which contain 0.5-10 mega bases of DNA [77]. Hereditary CRC syndromes are as a result of germline mutations while sporadic CRC is by alteration of DNA structure (mutation) or DNA function (epigenetics) [78].

Chromosome Instability (CIN)

Chromosomal instability (CIN) is more common than microsatellite instability (MIN) and occurs in about 70% of human cancers [78]. This involves gains and losses of whole chromosomes known as chromosome missegregation and has an important role in tumourigenesis [77]. CIN is driven by activation of mutations in oncogenes such as *KRAS* and tumour suppressor genes such as *APC* or *P53* [78], which results in aneuploidy, the presence of an abnormal number of chromosomes in a cell and a cause for tumorigenesis [79,80] (Figure 3). An example of a gene that follows the CIN pathway is *hBUB1*, a component of the mitotic spindle checkpoint (MSC), which is responsible for controlling mitotic checkpoints and chromosome segregation. Loss-of-function mutations of the *BUB1* gene results in aneuploidy [81]. Aneuploidy leads to additional chromosome missegregation and DNA damage, after which p53/p21 tumour suppressor pathway is triggered in order to limit further proliferation of aneuploidy cells. Nevertheless, when the *p53/p21* gene is mutated, this function becomes null and void [77,82]. Individuals with medical conditions characterised by aneuploidy such as Down syndrome and Turner syndrome have been found to show high incidence of cancer [83]. However, in CIN, aneuploidy found in the tumour cell population is heterogeneous and has the capability of selective evolution resulting in metastasis [82].

Studies have shown that CIN is responsible for tumorigenesis [84]. For instance, chromosome misseggregation was induced in mice by perturbing microtubule attachments to chromosomes. This led to the formation of lymphomas and lung tumours [85]. However, disruption or inactivation of the *P53/P21* pathway was required for aneuploidy karyotypes to survive because chromosome misseggregation in cells with a functional *P53/P21* system will lead to cell cycle arrest. CRC cell lines SW480, HRT-18, HT-29, DLD-1 have been found to possess mutation status at *P53* gene [86], which suggests that chromosomal misseggregation in CRC is mainly via the CIN pathway.

Microsatellite Instability (MIN)

Microsatellite instability (MIN) pathway results in about 5% of CRCs with the chromosome number not affected (diploid). It is caused by germline mutations in one of the DNA MMR (mismatch repair) genes, which are responsible for recognising and repairing errors that occur during DNA replication and also repair of DNA damage [87]. Mutations in MMR genes eventually leads to Lynch syndrome CRCs [88]. Mutations found in regulatory regions of the MMR genes *MSH2, MSH6, MLH1* and *PMS2*, which lead to MIN are one of the hallmarks ('Genome instability') for the development of CRC in humans [89,90,91,92]. These *mutL* and *mutS* homologues are six in number and all of which when deactivated as a result of mutation will lead to the development of cancer through the MIN pathway [93]. About 90% of mutations in Lynch Syndrome are caused by *MLH1* and *MSH2* mutations, with 7-10% by *MSH6* mutations, less than 5% by *PMS2* [94] and about 3% by *EPCAM* gene mutation [95]. The *EPCAM* gene is responsible for intercellular adhesion and intracellular signalling, migration and proliferation with its deletion leading to *MSH2* silencing [94]. MIN occurs in most patients with HNPCC [70] and is as a result of defects in the MMR genes, *mutS* homologue (hMSH2) and *mutL* homologue (hMLH1) [96] (Figure 4).

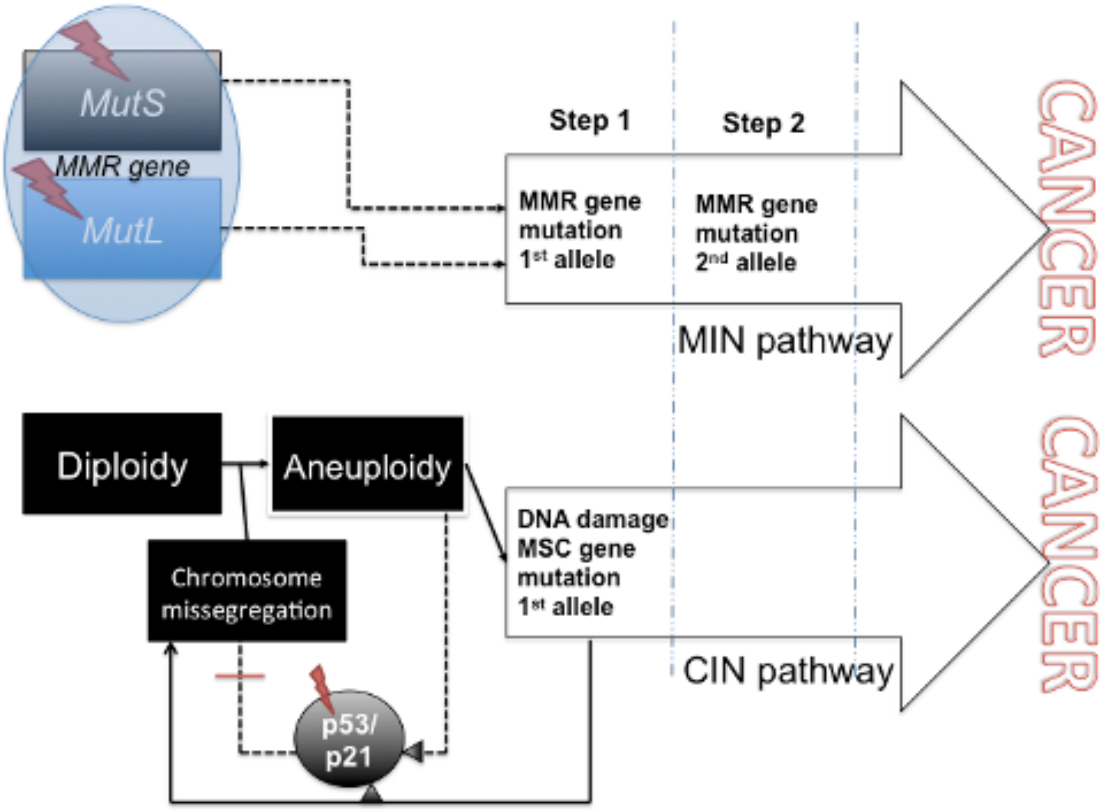

Figure 3: Different steps in the pathways to genetic instability and relationship between ploidy and CIN in terms of tumour initiation and growth. [Adapted from (Bakhoum and Compton, 2012, Lengauer *et al.*, 1998)]

CpG island Methylator Phenotype (CIMP)

CpG island methylator phenotype (CIMP) is also known as the serrated pathway, which results in CRCs that are DNA MMR deficient (MSI) or DNA MMR proficient (MSS), and make up about 25% of CRCs. The CIMP phenotype can be grouped into CIMP-high and CIMP-low with the BRAF oncogene mutation often found in CIMP-high CRC characterised by increased cell proliferation, progression of carcinogenesis [97]. Unlike CIN and MIN pathways, CIMP does not involve the activation of *KRAS* mutations [98]. However, in some contexts, *KRAS* mutations are found in CIMP CRCs and referred to as CIMP-low [99].

CRCs that are formed via MIN and CIMP pathways may be more difficult to diagnose in comparison to those that are formed via CIN pathway because they are commonly found in the proximal region of the colon, they are not detected easily by endoscopy and may develop quickly into cancer [100].

Treatment of CRC

Prognosis for patients with metastatic CRC (mCRC) is still poor [51]. In CRC, when detected early (while still localized), surgical removal may be curative. However, the early detection happens in only 39% of cases [101] and chemotherapy is often the only option.

Chemotherapy

Up until 1996, 5-fluorouracil (5-FU)-based therapy was the only therapy used in mCRC. Irinotecan, a topoisomerase inhibitor, was then approved that year and the platinum compound, oxaliplatin in 2004. A combination of 5-FU, leucovorin (folinic acid) with irinotecan is termed FOLFIRI while the combinations of 5-FU and leucovorin plus oxaliplatin is termed FOLFOX [101]. Capecitabine (Xeloda) is converted to 5-FU in the body and in combination with oxaliplatin is termed XELOX. A combination of Capecitabine and FOLFIRI is known as XELORI (CRUK). To further improve chemotherapy regimens, monoclonal antibodies; bevacizumab, cetuximab and panitumumab were then introduced as combinations with FOLFIRI and FOLFOX. Cetuximab may be administered alone for patients intolerant to irinotecan [101,102] (Figure 4).

In Nigeria, the same regimen is used for mCRC treatment [103], even though, the pathological

profile of CRC in indigenous Africans appears to be different and thus treatment protocols to accommodate such differences strongly suggested [104]. The heterogeneity of CRC is a worldwide phenomenon, hence, a lot of research and compilation of large databases on genome profiling, which has made therapy more precise, thus improving prognosis [105].

Oxaliplatin in combination with 5-FU and Leucovorin (Figure 4) resulted in an improvement in disease-free survival of patients from CRC and a 23% relative reduction in the risk of recurrence [106].

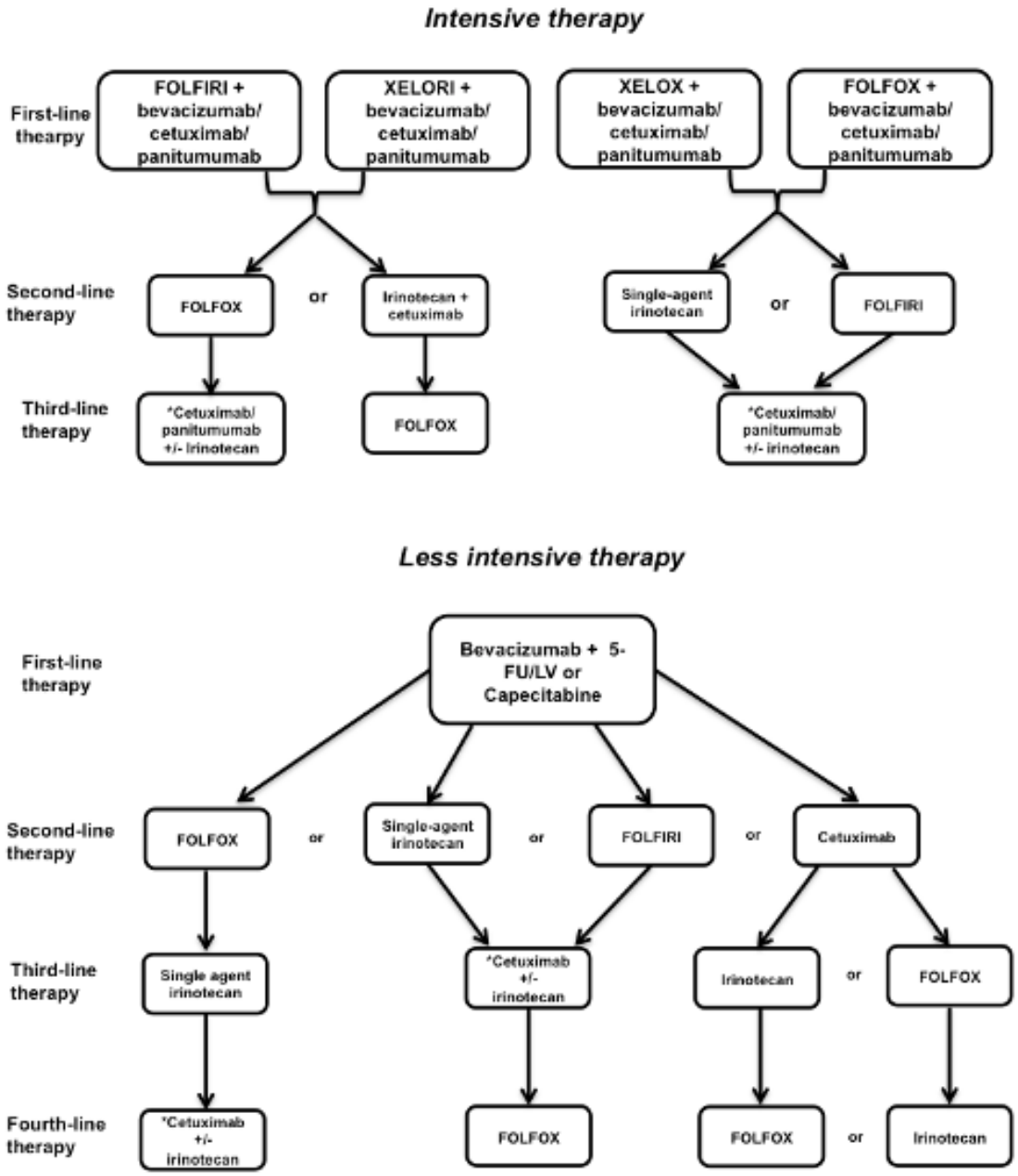

Figure 4: Intensive and less intensive treatment lines for mCRC [Adapted from (Alatise *et al*., 2017 and Goldberg *et al*., 2007)]

Peripheral neuropathy is the main safety concern associated with oxaliplatin. Frequent, transient, distal paresthesias are induced by oxaliplatin shortly after administration [106]. Lean body mass (LBM) have been found to be a significant predictor in toxicity and neuropathy in patients undergoing FOLFOX therapy such that patients with low LBM are susceptible to oxaliplatin toxicity, which has led to a cut off dose point of 3.09 mg oxaliplatin/kg LBM. It is therefore essential to use conventional

body surface area dosing in order to reduce such serious side effects [107].

Immunotherapy

The new trend in CRC therapy however is the emergence of immunotherapy, which is as a result of decades of research and the understanding of the relationship between the immune system and cancer. Immunotherapy has the ability to cause the immune system to attack and destroy cancer cells by recognising specific antigens that are found on tumour cells. Research has led to the development of therapies approved by the FDA such as a cancer vaccine in 2010 known as sipuleucel-T and immunomodulatory antibodies, iplimumab in 2011, nivolumab and pembrolizumab [51,108,109]. Various success stories about the use of immunotherapy in the treatment of cancer have been published. Such treatments include T-cell transfer therapy involving tumour-infiltrating lymphocytes (TIL) consisting of CD8+ T-cells obtained from a patient with mCRC which were able to recognise cancer cells harbouring mutant *KRAS* G12D gene [110].

Carcinoembryonic antigen (CEA) is the most widely studied tumour associated antigen (TAA) in CRC and has been on-going since the 1960s [111]. CEA is a plasma membrane-associated glycoprotein expressed by adult tissues and overexpressed by adenocarcinomas of the colon, breast lungs, which can be found in the serum [112]. Another TAA targeted in immunotherapy is MUC1 (mucin), a transmembrane glycoprotein, found on the surface of secretory epithelial cells which protects the body from bacterial invasion by binding to pathogens [113]. The overexpression of MUC1 in CRC regulates β-catenin and Ras tumour promoting signalling pathways leading to poor prognosis [114]. An adenoviral gene delivery platform encoding CEA antigen, *Ad5* has been shown to cause cell mediated immunity in over 60% of patients with advanced stage CRC [49].

A. Cancer vaccines

Cancer vaccines stimulate the immune system in to recognising tumour-specific antigens harboured by cancer cells as foreign bodies. This facilitates the attack of cancer cells by the immune system [51]. Whole-cancer-cell immunotherapies have a promising future in the treatment of CRC with OncoVAX®, a personalized cancer vaccine that uses the patient's own cancer cells to prevent tumour progression after surgery, in its confirmatory Phase IIIb clinical trials [115]. Cancer vaccines can be categorised into autologous tumour cell vaccines, dendritic cell vaccines, DNA vaccines and viral vector-based vaccines [50].

B. Cytokine therapy

Studies on the use of cytokine therapy in CRC is quite limited [51]. PEGylated recombinant IL-10 (AM0010) in a phase I study resulted in a sustained systemic Th 1 immune stimulation with a CRC patient having the cancer under control for over 40 weeks [51].

C. Toll-like receptors (TLR) agonists

Toll-like receptor (TLR) agonists act by targeting the innate immune system [116] with TLR-9 out of the ten human TLRs (TLR-1 to TLR-10) exhibiting protective role against malignant transformation in colorectal mucosa [117]. Examples of TLR agonists in early clinical trials include CpG-oligodeoxynucleotides (CpG-ODN) and MGN1703, a synthetic DNA-based immunomodulator with a dumbbell-like structure, which are being designed as maintenance therapy in mCRC patients after standard first-line therapy [51].

D. Adoptive cell transfer (ACT)

Adoptive cell transfer (ACT) involves the harvesting of autologous T-cells from TILs, activating and expanding them in number *ex vivo* after which they are then re-introduced into the patient host. This method of introducing large numbers of T-cells has been beneficial in tumour regression [118]. This method of therapy is particularly attractive because it targets driver mutations, which are tumour-specific and may likely be harboured by all the tumour cells [119]. Such mutations include the *KRAS* oncogene with reports of *KRAS* G12D expressed in about 13% of CRCs [120]. A patient that received TILs consisting of about 75% CD8+ T-cells reactive to KRAS G12D, showed regression of all seven metastatic lung lesions present in the patient after 40 days of therapy with only one of the lesions progressing after 9 months [110]. However, limitations in this therapy include lack of immune memory, protracted time and cost of T-cells production and risk of severe side effects [50].

E. Checkpoint inhibition

Checkpoint inhibitors are monoclonal antibodies that deregulate the major histocompatibility complexes T-cell Receptor signalling pathways by targeting co-inhibitory molecules that are responsible for the suppression of the immune system via stimulating T-cell dysfunction or

apoptosis [121]. These co-inhibitory molecules include CTLA-4, PD-1, PD-L1/2, lymphocyte-activation gene-3, T-cell immunoglobulin, T-cell immunoglobulin mucin-3 [51].

a. CTLA-4 (Cytotoxic T-lymphocyte associated protein-4) inhibitors

CTLA-4 is a receptor found on the surface of CD4 and CD8 T cell membranes responsible for inhibiting the activation of the immune system. Examples of CTLA-4 inhibitors include ipilimumab and tremelimumab used for melanoma [51]. These inhibitors however have not been successful in CRC therapy [122].

b. PD-1 (Programmed death-1) inhibitors

PD-1 receptor induces exhaustion in effector T-cells [52]. PD-1 inhibitors such as pembrolizumab block the interaction between PD-1 receptor and its ligands, PD-L1 and PD-L2 in order to enable T-cell activation and subsequent antitumor immune response [53]. About 50% of CRCs express PD-L1 [123]. Pembrolizumab has been shown to be effective in patients with MSI (DNA MMR deficient) and also in a patient with MSS (DNA MMR proficient) CRC [54,124].

ASPIRIN

Aspirin, a non-steroidal anti-inflammatory drug (NSAID) is an old drug commonly used for pain, inflammatory conditions and fever therapy [125]. Its active metabolite is known to be salicylic acid, which was used over two centuries ago by a Greek physician as extracts of the willow bark to treat fever [126].

Pharmacology of Aspirin

Several modes of action for aspirin as an antiproliferative and chemopreventative agent have been suggested (Figure 5).

i. The accepted pathway by which aspirin acts as an anti-inflammatory agent is irreversible cyclooxygenase inactivation through non-enzymatic acetylation of a single serine residue, which ultimately leads to the inhibition of prostaglandin biosynthesis [127,128,129,130].
ii. The inhibition of I-kappa kinase (IκK), thereby preventing the activation by NF-κB and its ability to regulate gene expressions that cause antiapoptosis in cancer cells [129,131,132,133].
iii. Inhibition of the JNK pathway [134].
iv. Activation of AMP kinase (AMPK) [135].
v. Aspirin has been found to cause apoptosis through the Wnt-β-catenin pathway [136]. Inhibition of the Wnt/β-catenin pathway targets gene expression involved in tumorigenesis when β-catenin interacts with T-cell factor (Tcf) in the nucleus.
vi. Normalising EGFR expression [137].
vii. Increase in the expression of hMLH1, hPMS2, hMSH2 and hMLH1, which are DNA MMR proteins [138].
viii. Inhibition of VEGF, which leads to the suppression of angiogenesis [139,140].
ix. Inhibition of the binding of c-Raf to Ras resulting in the inhibition of extracellular signal-regulated protein kinase (ERK) pathway, which is involved in various downstream signals that cause proliferation, cell differentiation and cell survival [129,132].

The general pharmacology of aspirin and other NSAIDs is known to be the inhibition of COX-1 and COX-2 (cyclooxygenase) (Figure 5) to prevent the generation of prostanoids from arachidonic acid. These prostanoids, which are biologically active lipid mediators, play significant roles in many physiologic pathways and include prostaglandin PGD_2, PGE_2, $PGF_{2\alpha}$, prostacyclin and thromboxane (TX) A_2 [132]. Prostanoids have an effect in various health conditions such as inflammation, pain, asthma, platelet function, renal function, cardiovascular homeostasis and cancer [141].
It has been shown that repeated doses of aspirin at a low dose (75-100 mg/day) causes maximal inhibition of platelet COX-1 activity after several days without greatly affecting cells elsewhere in the body. This effect lasts to about 24 hours [132]. Aspirin is primarily absorbed in the stomach when taken orally and goes through the GI tract and hepatic first-pass metabolism [142]. Aspirin undergoes hydrolysis by non-specific esterases and inhibits COX by acetylation before it is absorbed into the system and thus the reason for reduced systemic levels of aspirin and high levels of salicylic acid which is not as potent [132].

Chemoprevention with Aspirin

Chemopreventative and chemotherapeutic effects of aspirin in cancer have been widely studied [143] and it has been found to reduce the risk of CRC.
Despite a population-based cohort study that concluded non-aspirin NSAIDs to have a greater

effect than aspirin in reducing the risk of CRC [144], over the years a number of randomised controlled studies have been carried out which have suggested a protective effect or reduced risk against colorectal adenoma/cancer with the regular use of aspirin [145,146,147,148,149,150,151], which also led to the recommendation of low dose aspirin for the prevention of CRC by the United States preventative services task force [152].

An analysis of individual patient data from three large UK trials based on daily aspirin versus non-aspirin with approximately four years duration of treatment was the first proof in man that indeed aspirin reduced mortality from several cancers [153]. Aspirin has a dose-dependent effect on CRC risk with probably the highest risk reduction in patients taking a low dose after five years of continuous use [154].

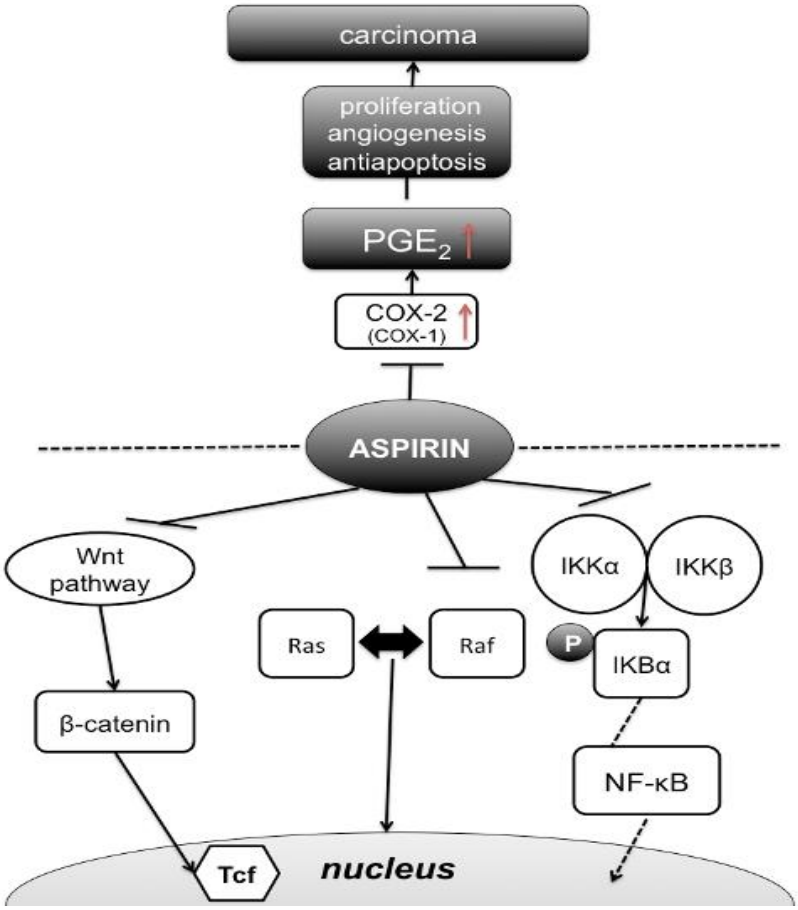

Figure 5: COX-dependent and COX-independent mechanisms of antitumoural effects of aspirin [Adapted from (Bruno *et al.*, 2012, Schror, 2011)].

Risks associated with aspirin therapy

There is increasing evidence of the benefits of aspirin in the prevention of CRC and cardiovascular events. However, it still presents with some health risks such as gastrointestinal (GI) toxicity, in particular GI bleeding and peptic ulcer, which have been found to be mostly age-dependent [155,156] and rarely fatal [157].

Data from randomised, controlled, trials have shown there to be about a two-fold increase in the risk of GI complications [157] regardless of the aspirin being in enteric-coated or buffered formulation [144]. However, administration of proton-pump inhibitors in combination with aspirin greatly reduced these GI effects [158,159].

The symptoms of GI complications are made worse in the presence of *H. pylori* infection even though it does not initiate or predispose to NSAID gastropathy [160]. It is thus recommended for patients to undergo *H. pylori* screening before

commencing on aspirin therapy to reduce the possibility of GI side effects.

CONCLUSION

Further understanding of the prevalence of CRC nationwide and possible mechanisms of action for aspirin in the disease gives hope for a more affordable, less toxic therapy for the prevention, treatment and management of cancer. In order to achieve this, more research should be encouraged in this area. Aspirin being adapted in Nigeria as a chemopreventative drug in high-risk and CRC patients that have completed chemotherapy will also help in data collection to find out if aspirin also has an effect in indigenous African patients.

CONFLICT OF INTEREST

The authors alone are responsible for the content of this research and report no conflict of interest

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