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Therapeutic Approach to KRAS Mutated Colorectal Cancer

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Introduction

A deeper understanding of the biology driving cancer has helped shape treatment approaches. Cancer therapy options have consistently moved away from typical cytotoxic chemotherapy where patients with a given cancer were treated equal, to an individualized approach where a tumor is defined by its genetic profile, pertaining to protein expression and gene mutations [1].

Throughout the years, treatment options for Colorectal Cancer (CRC) have improved dramatically due to the immense research being conducted worldwide. These studies have guided the medical professionals to have better insight of the disease at molecular levels leading to better targeted therapeutics. Cancer is progressively becoming appreciated to be caused by not only genetic alterations, but also by epigenetic alterations such as post-translational modifications. Increasing evidence is indicating that post-translational modifications play an important role in cancer progression and maintenance. Overall survival for patients with metastatic colorectal cancer has improved over the past decade [2].

Malignant tumors produce enzymes that allow them to invade other tissue, they often spread to new locations, a process known as metastasis. In this process, one or more of the transformed cells lose their attachment to the other cells of the tumor, break through the basement membrane, and spread via the circulation to other areas of the body. In the new location they regain attachment and continue to divide, forming new tumors. The new tumors are of the same type as the original tumor and thus when viewed with a microscope are seen to be different from the cells around them. Cancers that have begun to metastasize are far more serious and more resistant to treatment than those that have not, particularly because surgery cannot eliminate all the cancerous cells that have spread.

Cancer becomes lethal when cells metastasize, a multifactorial process of malignant cells spreading throughout the body from the primary tumor site. Halting the metastatic progression has been the predominant area of focus that has proven to increase the survival rate of patients with CRC. The activation and deactivation of cell signaling pathways that induce or dissuade

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the transcription of proto-oncogenes and tumor suppressor genes are being constantly explored by researchers. Among the many identified modulators, those which play pivotal role in transcriptional and translational regulations has been dissected meticulously.

One of the significant contributors of morbidity and mortality in western populations is colorectal cancer. This cancer initiates with the pathologic transformation of normal colonic epithelium to an adenomatous polyp and ultimately an invasive cancer [3]. It often takes several years to over a decade for the multistep disease progression and is accompanied by specific genetic alterations. According to the epidemiology of CRC, the disease manifests itself in three forms: family, hereditary, and most commonly sporadic, apparently not associated with any hereditary or familial factor. For the types having inheritance patterns and a family predisposition, the tumors develop through defined stages ranging from adenomatous lesions to the manifestation of a malignant tumor [4]. It has been documented that environmental and hereditary factors significantly affects the development of colorectal cancer, as indicated by the accumulation of mutations in oncogenes, genes which suppresses the repair of the DNA, signaling the existence of various pathways [4]. Furthermore, alternate pathways through mutation in BRAF and KRAS genes are also associated with the progression of polyps to cancer. Mutations in two classes of genes, tumor-suppressor genes and proto-oncogenes, are thought to impart a proliferative advantage to cells and contribute to the development of the malignant phenotype [3]. Inactivating mutations of both copies (alleles) of the Adenomatous Polyposis Coli (APC) gene--a tumor-suppressor gene on chromosome 5q--mark one of the earliest events in colorectal carcinogenesis [3].

Hereditary colorectal cancers

The hereditary conditions associated with an increased risk of colorectal cancer include well-characterized autosomal dominant syndromes, such as Familial Adenomatous Polyposis (FAP) and Hereditary Nonpolyposis Colorectal Cancer (HNPCC) [5]. Germline mutation of the APC gene followed by the mutation of the second APC allele is determined to be the cause of inherited familial adenomatous polyposis syndrome [4]. This syndrome is characterized by the presence of hundreds to thousands of colonic adenomatous polyps. If these polyps are left untreated, colorectal cancer develops over time. Patients with the classic type of familial adenomatous polyposis often begin to develop multiple noncancerous polyps in the colon by their teenage years, the number of polyps increases with age, and hundreds to thousands of polyps can develop on the colonic epithelium. Prior to the onset of malignant transformation, the noncancerous fibrous growths are called desmoid tumors. Desmoid tumors tend to recur after they are surgically removed. All the same surgical removal of the polyps and desmoid tumors is essential to avoid the development of malignancy. The average age for classical FAP development is at around 39 years. A variant of the disorder, called attenuated familial adenomatous polyposis, has delayed polyp growth with an average age of colorectal cancer onset around 55 years of age [6].

In both classic familial adenomatous polyposis and its attenuated variant, benign and malignant tumors are sometimes found in other places in the body, including the duodenum. The disease with colon polyps as well as growths outside the colon are categorized as Gardner syndrome [7]. A milder type of familial adenomatous polyposis, called autosomal recessive familial adenomatous polyposis, has also been identified. Pa-

tients with the autosomal recessive type of this disorder have fewer polyps than those with the classic type. Fewer than 100 polyps typically develop, rather than hundreds or thousands. The autosomal recessive type of this disorder is caused by mutations in a different gene than the classic and attenuated types of familial adenomatous polyposis [8,9]. A novel autosomal recessive form of FAP, caused by mutations in the base excision repair gene MYH, has recently been documented and provides further evidence for the importance of DNA repair mechanisms in CRC development, already documented by the involvement of the mismatch repair in HNPCC. Additional CRC-predisposing conditions, such as hyperplastic polyposis and hereditary mixed polyposis syndrome, are also being outlined. Heterogeneity of genetic mechanisms has important consequences in hereditary CRC. Nevertheless, classical mendelian conditions represent only a minor share of the total CRC population burden with low penetrance [5].

Frequent mutations in colorectal cancer

Recent studies have examined colorectal carcinogenesis in the light of several other genetic alterations. Mutation leading to dysregulation of the KRAS protooncogene is an early event in colon cancer formation. Conversely, loss of heterozygosity on the long arm of chromosome 18 (18q) occurs later in the sequence of development from adenoma to carcinoma, and this mutation may predict poor prognosis [10]. Loss of the 18q region is thought to contribute to inactivation of the DCC (deleted in colorectal carcinoma) tumor-suppressor gene. This gene encodes a transmembrane protein and was characterized in 1990. It is found to be epigenetically silenced in CRC thus promoting the disease progression [11]. More recent evidence suggests that other tumor-suppressor genes--DPC4 (Mothers against decapentaplegic homolog 4) a highly conserved SMAD family of transcription factor proteins that act as mediators of TGF- β signal transduction and MADR2 (Mothers against decapentaplegic homolog 2) a substrate for the receptor of the transforming growth factor beta (TGF-beta) pathway--also may be inactivated by allelic loss on chromosome 18q [3]. In addition, mutation of the tumor-suppressor gene p53 on chromosome 17p appears to be a late phenomenon in colorectal carcinogenesis. This mutation may allow the growing tumor with multiple genetic alterations to evade cell cycle arrest and apoptosis. Neoplastic progression is probably accompanied by additional, undiscovered genetic events, which are indicated by allelic loss on chromosomes 1q, 4p, 6p, 8p, 9q, and 22q in 25% to 50% of colorectal cancers [3]. The DNA repair genes for the third category of genes whose inactivation has been implicated in tumorigenesis of colorectal cancer. It has been reported that DNA mismatch repair deficiency, due to germline mutation of the MSH2, MLH1, PMS1, or PMS2 genes, contributes to development of hereditary nonpolyposis colorectal cancer (HNPCC) [12]. The majority of tumors in patients with HNPCC and 10% to 15% of sporadic colon cancers display Microsatellite Instability (MSI), also known as the replication error positive (RER+) phenotype. This molecular marker of DNA mismatch repair deficiency may predict improved patient survival. Mismatch repair deficiency is thought to lead to mutation and inactivation of the genes for type II TGF-beta - receptor and insulin-like growth factor II receptor [3]. Telomere shortening is an early event in CRC carcinogenesis and telomere/telomerase dysfunction is considered as a fundamental player in this process [13]. The level of telomere-specific reverse transcriptase (hTERT), the catalytic component of the telomerase complex, increases along with CRC progression. For telomerase, there is a consensus that high telomerase

activity is often associated with poor prognosis [14]. Several other genetic alterations promote the transformed colorectal epithelial cells to escape cell cycle arrest or apoptosis and have been concurrently recognized. In addition, the CpG Island Methylator Phenotype (CIMP) pathway exhibits gene silencing due to a widespread hypermethylation of CpG islands at several loci and thus hypomethylation or hypermethylation of DNA sequences may alter gene expression without nucleic acid mutation [15]. Individuals at high risk for hereditary colorectal cancer should be offered genetic counseling, predictive molecular testing, and when indicated, endoscopic surveillance at appropriate intervals [3].

KRAS mutation

KRAS mutations are the most frequent and prevalent driver oncogenes in various cancers, occurring in 25-30% of cancer patients. *KRAS* is a proto-oncogene that encodes a small 21-kD guanosine triphosphate (GTP)/ Guanosine Diphosphate (GDP) binding protein involved in the regulation of the cellular response to many extracellular stimuli [16]. *KRAS* is located at 12p12.1, spans approximately 38 kb, and encodes a 188-amino acid residue with a molecular weight of 21.6kDa. *KRAS* normally functions in signal transduction cascades initiated by the binding of the Epidermal Growth Factor Receptor (EGFR), hepatocyte growth factor, and insulin-like growth factor to their receptors [17]. When activated, wild-type *KRAS* binds GTP, triggering a conformational change that allows the protein to bind and activate over 20 known downstream effectors, including Raf, Braf, mTOR, MEK1 and 2, ERK, AKT, and PIK3CA. These downstream effectors exert many different effects, including apoptosis suppression, promotion of cell growth, cell transformation, angiogenesis, migration, and differentiation [18]. The GTPase *KRAS* switches between its inactive (GDP-bound) and active states (GTP-bound). *KRAS* is frequently mutated in human cancers in the pancreas, lungs, and colon. 99.2% of the mutations are most abundant at the 12th, 13th and 61th residue position [19]. About 30% of human cancers account for point mutations in the *KRAS* gene, particularly in adenocarcinomas of the pancreas, lung, and colon.

Oncogenic *KRAS* mutants are prevalent at positions 12, 13, and 61 in cancer patients. The most common substitutions are for glycine at the 12th and 13th positions for G12D (33.7%), G12V (32.7%), G12C (14%), and G13D (12.5%). However, G12X is most frequently mutated (89%). G12 is the location of the protein's active site, consisting of a p-loop (residues 10-17) along with two switch regions (SI, residues 25-40 and SII, residues 60-74). Mutation at position G12X leads to bulkier side chains in the active site, which interferes with steric activity in GTP hydrolysis [19]. This mutation, G12X, is associated with detrimental oncogenic properties due to deficiency of intrinsic GTPase activity. Active WT *KRAS* plays a role as a GTPase through hydrolysis of the gamma phosphate in GTP, bringing it to an inactive state (GDP) [19]. Mutant *KRAS* however, impairs GTPase activity, preventing hydrolysis of the gamma phosphate. Inability to switch to a GDP-bound state, mutant *KRAS* continues in its active state, leading to continuous downstream signaling associated with oncogenic cellular growth.

Treatment modalities of *kras* mutated colorectal cancer

Metastatic Colorectal Cancer (CRC) remains an incurable illness with a median survival time of approximately 2 years. A subgroup of CRC patients (~45%) harbor mutations in the *KRAS* oncogene and are precluded from receiving the anti-EGFR tar-

geted therapies [20]. In 2020, CRC was responsible for 53,200 deaths, 816,000 life years lost, and a staggering \$ 9.4 billion in economic costs [21]. Despite tremendous financial investment and research effort, the 5-year survival rate for patients with advanced/ stage IV/metastatic(m) CRC is only 15% [22]. The lack of alternate treatments for these patients makes this an area of urgent investigation and unmet medical need. Among patients with stage IV cancer, a mutation in the *KRAS* oncogene is prevalent in 45% of tumors [23-25]. Decades of research has identified *KRAS* mutation as a negative predictive biomarker of Anti-Epidermal Growth Factor Receptor (EGFR) therapy (cetuximab and panitumumab), and such patients are excluded from these therapies [23,24]. These drugs target EGFR, and downregulate the Mitogen Activated Protein Kinase (MAPK) pathway, of which RAS is a key intermediate step. In tumors with an oncogenic *KRAS* mutation, the RAS protein is locked in a GTP bound state, and is constitutively activated, leading to downstream activation of the MAPK pathway [26]. In such a situation, inhibiting this pathway proximal to the RAS protein, as is the case with the anti EGFR antibodies, is redundant, directly leading to tumor resistance. Currently, there are no therapeutic agents directly targeting the *KRAS* pathway partially due to the complexity of network and multitude of downstream collateral pathways. Thus, finding adjuncts to the current treatment arsenal is challenging, yet vital [27]. Thus, designing a therapeutic strategy for the *KRAS* mutated CRC patient cohort is urgent as the cohort has limited options of FDA- approved therapies.

Oncolytic virus

Important research in the past decade has revealed unique viral characteristic not registered in any other forms of micro-organism [28]. Throughout the process of pathogen-host co-evolution, viruses have developed a battery of distinct strategies to overcome the very sophisticated defense mechanisms of the infected host [19]. The search for oncolytic virus roots from the fact that transient cancer remission can occur following viral infections [19]. One of the important members of the therapeutically identified virus family is the respiratory, enteric, orphan virus commonly known as reovirus. An exciting characteristic of reovirus is that it is naturally oncolytic. Reovirus has an inherent propensity to replicate in cells harboring dysfunctional growth factor signaling cascade that includes *KRAS* activation [29]. *KRAS* activation is notorious in cancer scenario most prominently in pancreas followed by colon, thyroid and lungs [30]. Reovirus type 3 Dearing strain is a naturally occurring, ubiquitous, non-enveloped human double-stranded (ds) RNA virus. It is purported to replicate selectively in transformed cells with an EGFR pathway induction or *KRAS* mutation [31,32], or with a v-erb B oncogene [33]. In non-transformed cells, the early viral transcripts lead to the auto-phosphorylation of dsRNA-activated Protein Kinase R (PKR). The activated phosphorylated PKR in turn phosphorylates and activates the alpha subunit of the eukaryotic translation initiation factor 2 and subsequently inhibits viral protein synthesis [34]. In transformed cells, the active *KRAS* signaling pathway inhibits the auto-phosphorylation of PKR, and thereby permits the synthesis of viral proteins, facilitating the uninhibited replication of the virus [28]. Though the safety, feasibility, and potential efficacy of reovirus as a cancer therapy are currently being evaluated in phase I-III clinical trials, the underlying molecular mechanism by which reovirus preferentially induces oncolysis in *KRAS* mutant remains elusive.

It is now being increasingly appreciated that reovirus adopts a bimodal mechanism to destroy the cancer cell. The *KRAS* mu-

tated tumor cells have been found to be cold in nature with immune deserted tumor micro-environment. Reovirus along with its oncolytic arsenal can also exerts consequences of a typical viral infection where the infected tumor cells mount a strong immune response. This leads to the accentuation of the infiltrating T lymphocytes that can also act on tumor cells and kill them. At present research is underway to evaluate the immune stimulation properties of reovirus.

Reovirus and autophagy

The association between *KRAS* mutation and autophagy induction in cancer is complex. Several studies have shown that *KRAS* mutation induces autophagy, while others have reported inhibition [35,36]. A broad-spectrum study was recently instituted where in three different models were employed to assess the contribution of autophagic machinery toward efficient self-propagation and oncolytic activity of reovirus in *KRAS* mutated colorectal cancer [27]. This is one of the first studies evaluating the alterations in autophagic pathway in colorectal cancer secondary to reovirus intervention, in which we focused on *KRAS* mutation, as reovirus is significantly effective under *KRAS* mutated condition. The study clearly reveals that several important autophagic proteins are significantly upregulated in *KRAS* mutant as compared with the *KRAS* WT conditions. Autophagy can be associated with both cancer progression and tumor suppression and can promote cell survival or activate programmed cell death. As an outcome of the study, it is predicted that administration of reovirus in conjunction with autophagy-activating drugs will synergistically improve the efficacy of reovirus and can be used as a dedicated therapy for the *KRAS* mutated colorectal cancer patient population [27].

Immunotherapy

The latest addition to the treatment arsenal is immunotherapy, where the patient's own immune system is re-programmed to recognize and destroy the tumor cells. As our understanding of the molecular mechanisms driving response to immunotherapy improvement, trials are being designed with genetically defined patient selection criteria. Checkpoints are a normal immune system regulatory mechanism that prevents the immune system from straying beyond its usual targets and destroying healthy cells instead. Checkpoints occur when specific protein receptors on T cells bind with proteins expressed by the body's own normal cells, an interaction that puts halt on T cells and prevents them from attacking "Self" cells they encounter. Most types of cancers can exploit checkpoint interactions by disguising their cells as "Normal." They do so by expressing surface proteins that bind with T cell receptors, causing a checkpoint interaction that tricks the T cells.

Conclusion

One of the toughest scientific challenges has been the development of therapeutic strategy to harness the detrimental consequences of activating mutations of *KRAS* protein. No therapies specifically targeting *KRAS* mutations in cancer have yet been approved by the US food and drug administration. *KRAS* mutations are often associated with resistance to targeted therapies and poor outcomes in patients with cancer, and despite four decades of intense scientific effort no selective *KRAS* inhibitor has been developed effectively. Here in we have reviewed the different possible therapeutic approaches that is being currently tested and the modest success that has been achieved thus far.

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