Journal of Cancer Science & Therapy
ISSN: 1948-5956

Farghal et al., J Cancer Sci Ther 2017, 9:10
DOI: 10.4172/1948-5956.1000492

Research Article Open Access

Genetic Variations of Selected Genes Using Target Deep Sequencing in Colorectal Cancer Patients

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Abstract

Background: Colorectal carcinoma (CRC) is a burden problem in a developing country like Egypt since patients are usually admitted in late stage with bad prognosis and short overall survival. Because of genetic predisposition of CRC and introduction of advanced molecular techniques, efforts are directed to screen for potential pathogenic or disease-causing variants in CRC patients

Methods: DNA was isolated from formalin fixed paraffin embedded tissue sections collected from 24 CRC confirmed diagnosed patients. TruSight CRC panel (Illumina) was used for detection of different variants in 15 genes. The generated reads were obtained from Illumina Miseq were clustered into single nucleotide polymorphism (SNPs) and small insertions/deletions (Indels). Further pathogenic variants with somatic and germline mutations were identified according to the recommended criteria. Some CRC patients were subjected to anti-EGFR target therapy.

Results: Most of the variants were detected in *TP53* gene 140 variants (65%); 105 short deletions none of them was pathogenic, 29 missense mutations and 6 SNPs at splicing sites. Next, *ERBB2* has got 17 variants (8.8%) (missense and splicing), 8 of them were damaging disease causing variants. Besides, 16 pathogenic variants were identified in 12 patients (6 in *TP53* and 7 in *KRAS*). Some pathogenic variants were not reported before in CRC e.g. *TP53* C>A, rs121912654, Val157Phe. Additionally, patients carried different *KRAS* wild mutations showed variable response to anti-EGFR target therapy.

Conclusion: The most affected pathway in CRC was *TP53* pathway followed by *ERBB2, NRAS*, *KRAS* and *PIK3CA* genes. Variable response to target therapy suggested dependence on the type of pathogenic variant identified, also a possible role of *ERBB2* which had a significant variant frequency.

Keywords: Colorectal carcinoma; Trusight next generation sequencing; Molecular diagnosis

Introduction

Colorectal cancer (CRC) is considered the third most common tumour and the fourth leading cause of cancer-related mortality worldwide. Its incidence will be increased by 60% by the year 2030 [1]. At initial diagnosis, 25% of CRC patients present with metastasis and 50% will develop metastasis after diagnosis [2]. Many factors contributed to the development of CRC including genetic factors [3] and environmental factors such as diet and lifestyle [4].

Genetic alteration is a common feature of CRC ranging from small molecular changes such as point mutations or small indels to chromosomal copy number variation or translocation [5]. *RAS* status was mandatory to be examined before the use of anti-EGFR therapy as recommended by ESMO guidelines 2106. Also, patients carry *BRAF* mutations might not respond to anti-EGFR therapy [6]. Beside the *RAS*/*RAF*, *MAP* kinase pathway, *TP53* and the PI3K/Akt/mTOR signalling cascade are critical. The process of CRC development is an interplay between suppression of some tumor suppressor genes (TSGs) including the key TSG i.e. *TP53* and activation of some oncogenes such as *KRAS*, *NRAS*. The prevalence rate of *TP53* mutation in Arab population is 52.5% compared to 47.5% in matched Western population [7]. *TP53* mutations have roles in determining progression, invasiveness and also metastasis of CRC. So, CRC patients with mutant *TP53* have more progressive phenotype and poorer survival than those with *TP53* wild type [8]. The phosphatidylinositol 3-kinase/Akt/mammalian target of

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Received September 18, 2017; **Accepted** October 16, 2017; **Published** October 20, 2017

Citation: Farghal EE, Saied MH, Ghaith FM, Moussa GI, El-Sharnobi G, et al. (2017) Genetic Variations of Selected Genes Using Target Deep Sequencing in Colorectal Cancer Patients. J Cancer Sci Ther 9: 683-689. doi:10.4172/1948-5956.1000492

rapamycin (PI3K/Akt/mTOR) signalling pathway has pivotal roles in cell proliferation, apoptosis, survival and metastasis [9]. The effects of PI3K are mediated by AKT. One of the downstream targets of AKT is mTOR which controls angiogenesis. Activation of PI3K/Akt/mTOR has been reported in the development and progression of CRC [10].

Next-generation sequencing (NGS) technology has the advantage of parallel deep sequencing of genetic mutations in multiple genes in patients simultaneously [11]. Targeted sequencing is a good clinical application of NGS technology since it directed to increase the coverage of read depth of hotspot mutations through localizing the number of genes of interest examined with maintaining the number of bases sequenced [12]. Therefore, we aimed in this study to screen for potential pathogenic variants in Egyptian CRC patients in different stages of the disease.

Materials and Methods

Formalin fixed paraffin embedded tissues (FFPE) samples that were obtained from 24 CRC patients, who were referred to Tanta Main University hospital between January 2016 and June 2017. This study was approved by Tanta Ethics Committee of Faculty of Medicine. Informed consents were obtained from all patients. The age distribution of all patients ranged from 30 to75 years with a median age of 55 years. The clinicopathological data of patients are summarized in Table 1. DNA was extracted from FFPE tissue samples using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). The concentration of DNA was determined using the Denovix fluorimeter (AGBL, USA).

Trusight tumor 15 panel illumina CRC panel

We sequenced patient's DNA using the Trusight Tumor 15 panel (Illumina, San Diego, CA, USA) that contains 15 genes suspected to have roles in cancer predisposition; *TP53*, *AKT1*, *BRAF*, *EGFR*, *ERBB2*, *FOXL2*, *GNA11*, *GNAQ*, *KIT*, *KRAS*, *MET*, *NRAS*, *PDGFRA*, *PIK3CA* and *RET*. Twelve genes showed coverage of 95% (≥ 20 reads). In these genes, the mean read coverage was 93.5% of bases covered at ≥ 500 × exonic and essential splice sites regions covered at ≥ 20 reads. Only, *NRAS*, *RET* and *GNA11* showed coverage less than 95% (≤ 20 reads). For library preparation, Trusight panel DNA library preparation protocol (Illumina) was used [13].

Age (years)	
Median (range)	55.0 (30.0-75.0)
Gender	
Female	10 (41.7.0%)
Male	14 (58.3%)
Primary tumor location	
Right colon	11 (45.8%)
Transverse colon	3 (12.5%)
Left colon	3(12.5%)
Sigmoid colon	3 (12.5%)
Recto sigmoid colon	1 (4.2%)
Rectum	3 (12.5%)
Histology	
Conventional adenocarcinoma	21 (87.5%)
Signet ring cell adenocarcinoma	3 (12.5%)
Growth pattern	
Ulcerative	17 (70.8%)
Fungating	4 (16.7%)
Infilterative	2 (8.2%)
Caulifiower mass	1 (4.2%)
TNM stage	
I	0
II	12 (50%)
III	6 (25%)
IV	6 (25%)
Baseline CEA and CA19-9 (ng/mL)	
High	4 (16.7%)
Normal	20 (83.3%)
TNM: Tumor, Node and Metastases; CEA: carcinoembryonic antigen	

Table 1: Clinicopathological criteria of CRC patients.

Bioinformatic Analysis

The FASTQ files were generated using MiSeq Reporter (Illumina). The FASTQ files were assessed for their base quality. More than 85% of reads showed base quality higher than 30. Next, reads were aligned to a reference genome hg19 and variants were called using Genome Analysis Toolkit (GATK). Then, VCFs were generated for further analysis by VariantStudio (Illumina). The passed filtered variants were selected for the analysis. Annotations included in VariantStudio were HGMD, ClinVar pathogenicity annotations, links to dbSNP, in addition to prediction tools such as SIFT, PolyPhen and Provean. We also used Varsome (The Human Genomic Variant Search Engine, https://varsome.com/), Genome Aggregation Database/exomes (gnomAD, ExAC) and Mutation Taster. The identified variants in this study were classified according to ACMG (American Society of Medical Genetics and Genomics) into five categories: (1) pathogenic, (2) likely pathogenic, (3) of uncertain significance (VUS), (4) likely benign and (5) benign [14].

Results

Pathogenic and likely pathogenic variants in the CRC cohort

Sixteen pathogenic and likely pathogenic variants were identified in 12 patients, which account for 50% of patients (Table 2). All of those variants were single nucleotide polymorphism (SNPs); 7 SNPs in *TP53* gene, 6 SNPs in *KRAS*, 1 SNP in *BRAF* and 2 SNPs in *PIK3CA* (Table 2).

***TP53* pathogenic and likely pathogenic variants:** Seven *TP53* pathogenic/likely pathogenic variants were identified in the studied patients. There were no repeated *TP53* mutations. Five out of seven *TP53* mutations were germline inherited. All of these mutations were missense mutations located in three exons; 5, 7 and 8 (the commonest sites of *TP53* mutations) [15]. Each exon has 2 identified mutations with one mutation found in exon 10. A pathogenic variant identified in patient ID 8 in exon 5 (C>A, rs121912654, Val157Phe), was not reported before in CRC, while it was found previously in patients with chronic lymphocytic leukemia [16] and hepatocellular carcinoma [17]. Similarly, a pathogenic variant was detected in exon 5 in patient ID 17 (C>T, rs28934578, Arg175His), is one of germline mutations detected in DNA binding domain in *TP53* in solid tumors e.g. breast cancer [18]. Patient ID 17 also has got another pathogenic mutation (G>A, rs587782529, Arg337Cys) outside the common sites of *TP53* mutations i.e. in exon 10. This mutation is associated with unclassical Li-Fraumeni syndrome [19]. Furthermore, exon 7 has got 2 mutations e.g. C>T, rs28934575, Gly245Ser which was previously described in CRC [20] and G>A, rs121912651, Arg248Trp which is a well-known pathogenic variant in CRC [21]. Exon 8 has got 2 pathogenic variants that are associated with Li-Fraumeni syndrome (G>A, rs149633775-Arg283Cys, C>T and rs763098116- Cys277Phe) [22]. Generally, *TP53* has got 29 missense mutations; 7 were reported as pathogenic, 3 with uncertain significance and the remaining missense mutations were benign/ likely benign.

Gene	Case no.	Exon no	Type of mutation	Variant	Heterogeneity	Inheritance	SIFT	Provean	Amino acid substitution	Age at diagnosis (Years)	Reference
TP53	17	Exon 10	SNP	G>G/A	Het	Germline	Damaging	Damaging	p.Arg337Cys	75	rs587782529
TP53	18	Exon 8	SNP	G>G/A	Het	N/A	Damaging	Damaging	p.Arg283Cys	48	rs149633775
TP53	5	Exon 8	SNP	C>C/T	Het	Germline	Damaging	Damaging	Cys277Phe	64	rs763098116
TP53	4	Exon 7	SNP	G>G/A	Het	Germline	Damaging	Damaging	p.Arg248Trp	55	rs121912651
TP53	1	Exon 7	SNP	C>C/T	Het	Germline	Damaging	Damaging	Gly245Ser	64	rs28934575
TP53	17	Exon 5	SNP	C>C/T	Het	Germline	Damaging	Damaging	p.Arg175His	75	rs28934578
TP53	8	Exon 5	SNP	C>C/A	Het	Somatic	Damaging	Damaging	Val157Phe	75	rs121912654
KRAS	10	Exon 4	SNP	C>C/T	Het	Germline	Damaging	Damaging	Ala146Thr	65	rs121913527
KRAS	20	Exon 3	SNP	G>G/A	Het	Germline	Damaging	Damaging	p.Thr58Ile	55	rs104894364
KRAS	3	Exon 2	SNP	G>G/A	Het	Somatic	Damaging	Damaging	Not applicable	62	rs397517040
KRAS	5	Exon 2	SNP	C>C/T	Het	Somatic	Damaging	Damaging	Gly13Asp	64	rs112445441
KRAS	16	Exon 2	SNP	C>C/T	Het	Somatic	Damaging	Damaging	Gly13Asp	50	rs112445441
KRAS	4	Exon 2	SNP	C>C/T	Het	Somatic	Damaging	Damaging	Gly12Asp	55	rs121913529
KRAS	11	Exon 2	SNP	C>C/G	Het	Somatic	Damaging	Damaging	Gly12Ala	48	rs121913529
KRAS	8	Exon 2	SNP	C>C/T	Het	Somatic	Damaging	Damaging	Gly12Ser	52	rs121913530
BRAF	7	Exon 15	SNP	A>A/T	Het	Somatic/Germline	Damaging	Damaging	Val600Glu	60	rs113488022
BRAF	19	Exon 15	SNP	C>C/A	Het	Somatic	Damaging	Damaging	Gly596Val	75	Not provided
PIK3CA	4	Exon 10	SNP	G>G/A	Het	Somatic	Damaging	Damaging	p.Glu545Lys	55	rs104886003
PIK3CA	16	Exon 21	SNP	A>A/G	Het	Somatic	Damaging	Damaging	p.His1047Arg	50	rs121913279

Table 2: Pathogenic and likely pathogenic variants in CRC patients.

***KRAS* pathogenic and likely pathogenic variants:** Six pathogenic variants/likely pathogenic variants were identified in *KRAS*, 4 mutations were in exon 2 (66%), one mutation in exon 3 and one mutation in exon 4 (Table 2). All of them were SNPs and somatically inherited. *KRAS* exon 2 mutations are found to be predictors of bad prognosis and resistance to anti-EGFR antibody therapy [23]. Pathogenic variant (C>T, 112445441, Gly13Asprs) was repeated in 2 patients; IDs 5 and 16. This mutation (Gly13Asprs) was associated with invasive pancreatobiliary tumors in Turkish population [24]. Pathogenic variant (rs121913529) was also identified in 2 patients; IDs 4 and 11. This mutation, in addition to the pathogenic variant (C>T, rs121913530, Gly12Ser) were detected in patient ID 8. Both variants were shown to discriminate conventional adenoma from CRC [25]. The remaining pathogenic *KRAS* variants were found in exon 3 (G>A, rs104894364, Thr58Ile patient ID 20) and in exon 4 (C>T, rs121913527, Ala146Thr patient ID 10), both are well-known pathogenic variants in CRC [26]. Furthermore, one pathogenic variant (A>T, rs113488022, Val600Glu) was identified in *BRAF* in patient ID 7, this mutation was found in sessile serrated polyps; precursor of CRC [26] (Table 2). Also, Patient ID 19 has got C>A, Gly596Val variant. Finally, two pathogenic variants were detected in *PIK3CA*; G>A rs104886003, Glu545Lys in patient ID 4, this mutation is one of the hotspots found in exon 10 that is considered a driver *PIK3CA* gene mutation [27]. Also, a known pathogenic *PIK3CA* mutation (A>G, rs121913279, His1047Arg) [21] was identified in patient ID 16. Therefore, most of pathogenic missense mutations were identified in *KRAS* in exon 2. Less commonly, few hotspot pathogenic variants were detected in *BRAF* and *PIK3CA*.

Missense, splicing and indels mutations

Next, total number of mutations in every gene was investigated. *TP53* has got the highest number of detected mutations in 24 patients i.e. 140 (65%); 105 short deletions none of them was pathogenic, 29 missense mutations and 6 SNPs at splicing sites, one of them was a disease-causing at intron 9 patient ID 22. *ERBB2* was the second most common gene that had mutations (8.8%). *ERBB2* has got 11 missense mutations: one in exon 17 (Arg678Trp), patient ID 8, four variants in exon 18; (Thr686Met) patient ID 7, (Glu717Asp) patient ID 14, (Ser728Phe) patient ID 3 and (Gly732Asp) patient ID 22. All of them were predicted as damaging by SIFT and Provean. However, three neutral tolerated missense variants in exon 20 were also identified in *ERBB2*: Val777Leu, Gly778Asp, Val777Leu and two tolerated neutral variants in exon 26 (Pro1121Leu, p.Val1128Ile). One missense variant predicted as damaging (Ser1054Ile) was identified in exon 26 in 3 patients IDs 4, 7 and 8. In addition, *ERBB2* has got 6 variants at splice site; one damaging variant (Pro699Ser) in exon 18 patient ID 1, one damaging variant (Pro1137Ala) in exon 26 patient ID 4. In addition, *NRAS* has got 6% of total identified variants. All ten missense mutations found in *NRAS* gene were of low frequency (seen in less than 10% of the reads covering a given base). Three low variant frequency mutations at the splice site were also identified; one in exon 3, two in exon 4; Gln99His patient ID 22 and Ser145Ter patient ID 19. Besides, *KIT* has got 3.8% of frequent detected variants; four missense mutations, three of them were predicted as damaging by SIFT and Provean; Gly432Val, Pro467Gln, Ser821Tyr, while a benign missense variant (Met541Leu) was identified. The latter mutation was repeated in patients IDs 1, 18, 22 and 2. In addition, *KIT* included four mutations at the splice site, one of them was predicted as damaging by SIFT and Provean in exon 17 (Asn828Lys) in patient ID 22. One variant at the splice site (exon 18, G>C, rs3733542, benign, germline) was repeated in patients IDs 1, 15, 16, 18, 21, 22 and 24. *PDGFRA* has also 3.8% of the identified variants; 6 missense mutations [Met578Ile, Asn656Lys, Gly838Asp, Ala840Thr, Asn659Lys, and Ala663Asp]. Besides, *PDGFRA* has a repeated inactivated silent SNP (A>G rs1873778, at codon 567 (P567P) in exon 12 [28] that presents as homozygous allele (GG) in all CRC patients except in patients IDs 6, 20, 22 where the variant was heterozygous (AG). The GG allele was the most frequent similar to European Caucasian population [29]. A characteristic insertion (T>TA) in exon 18 was also found in most of patients (homozygous TA/TA in patients IDs 1,2,4,7,8,9,10,11,13,14,15,16,17,22,23 and 24), (heterozygous T>TA, rs3830355, IVS18-50insA in patients IDs 3, 5,6, 12 and 18). Both *PDGFRA* exon 18 mutation and exon 12 mutation were related more to gastric and intestinal GIST (gastrointestinal tumors) more than to colonic [30]. Two splice site variants were also identified without provided amino acid substitution. Similarly, *KRAS* has got 8 identified mutations (3.8%); six of them were pathogenic as mentioned before and 2 intronic SNPs. *FOXL2* has 4 missense mutations Arg148Leu,

Arg147Cys, Arg145His, and Pro116Ser. All of them were in exon 1 and predicted as damaging mutations. Furthermore, *PIK3CA* showed 5 missense mutations with the following amino acids substitution: Arg524Lys, Glu542Gln, Glu545Lys, Met1043Val, and His1047Arg with one disease-causing nonsense mutation: Arg992Ter (Table 3). *BRAF* has 2 missense pathogenic variants (Val600Glu and Gly596Val) and another one at the splice site. *EGFR* has got 2 missense mutations that are predicted as damaging (Gly779Cys patient ID 22 and Val819Met patient ID 19), both of them were found in exon 20. Also, 2 variants were found in splice site in *EGFR*; one in exon 20 (Ala822Thr) and two in intron 17. *AKT1* has got only one variant at splice site: rs770565457, patient ID 17 with no clinical significance. Furthermore, two missense variants were predicted as damaging in exon 5 in *GNA11*; Arg210Trp in patients IDs 3, 19 and Arg213Trp in patient ID 3. Finally, *MET* gene has got two missense mutations; a tolerated neutral variant (Gly1102Asp) in exon16 patient ID 3 and a damaging variant (Pro1285Leu) in exon 20 patient ID 21. One variant at splice site was also found; (Asn1209Lys) in exon 18 patient ID 3. In summary, our analysis revealed that most of investigated variants were detected in *TP53* (65%) followed by *ERBB2* (8.8%), suggesting more involvement of *TP53* pathway than *KRAS* or *PI3K* pathways in Egyptian population [21].

TP53 showed the highest percentage of germline inherited mutations among CRC patients

Total of 37 mutations with known type of inheritance were identified in 24 patients. Out of these 37 mutations, 24 germline mutations were detected (65%), most of them were in *TP53* (10/24=42%) followed by *KIT* (5/24=21%). Thirteen somatic/acquired mutations were identified (35%). Most of the somatic mutations were found equally in *KRAS* (4/13=30%) and *EGFR* (4/13=30%). There was no significant correlation between the number of germline mutations in each patient with either lymph node metastasis (P=0.6) or distant metastasis (P=0.78). However, it was noticed that patient ID 4 has got the highest number of both germline mutations (5 mutations); 2 mutations in *TP53* and one mutation in each following gene *MET*, *PDGFRA*, *EGFR* in addition to 4 somatic mutations; 2 in *PIK3CA*, one in *KRAS* and one in *EGFR*. Patient ID 4 showed the highest number also of lymph node (LN) involvement (18 LNs) with distant metastasis in liver. No CRC patients who were involved in the current study had positive family history of malignancy except patient ID 14 who was the only one who had family history of uterine, colon and breast cancer. She was 50 years at the diagnosis of CRC with 2 lymph nodes involvement with liver metastasis. Interestingly, this patient did not have a known germline pathogenic variant, but she has got a missense mutation in *FOXL2* (G>A, Pro116Ser). This mutation was predicted as damaging by SIFT and Provean, and was not previously described in CRC. Family screening of this variant is recommended for that patient since this missense variant could be exist but with much less allelic frequency [31]. Patient ID 14 has got another damaging missense mutation in *ERBB2* in exon 18 (G>T, Glu717Asp); this mutation could be an activating mutation that is accompanied with *ERBB2* overexpression [32]. Another interesting finding was related to patient ID 9; who was the only case with bone marrow metastasis. Since bone metastasis is extremely rare in CRC [33], we searched for associated variants that might predispose to this sign. No pathogenic variants were detected, besides no characteristic variants were unique to patient ID 9. For instance, G>A, rs1050171 was identified in exon 20; this genotype is a predictor of bad responsiveness to anti-EGFR therapy in metastatic CRC [34]. Also, C>T, rs41736 in exon 20 in *MET* was also found but with no previous association with bone metastasis (Figure 1) Therefore, cytopathological examination from bone metastasis is very recommended since it is expected to have distinct mutations from that of primary tumour [35]. Concisely, most of mutations detected were germline (65%), commonly present in *TP53* (42%) while the somatic mutations (35%) were found mainly in two oncogenes i.e. *KRAS* and *EGFR*

Variants	GNA11 (n=2)	KRAS (n=7)	RET (n=1)	KIT (n=8)	AKT1 (n=1)	NRAS (n=13)	FOXL2 (n=4)	BRAF (n=2)	PIK3CA (n=6)	PDGFRA (n 8)	MET (n=3)	GNAQ (n=0)	EGFR (n=4)	ERBB2 (n=17)	TP53 (n=140)
Deletion	0	0	0	0	0	0	0	0	0	0	0	0	2	0	105
Missense	2	6	0	4	0	10	4	2	6	6	2	0	2	11	29
Splicing	0	1	1	4	1	3	0	1	0	2	1	0	2	6	6

Table 3: Missense, indels, splicing variants identified in CRC patients.

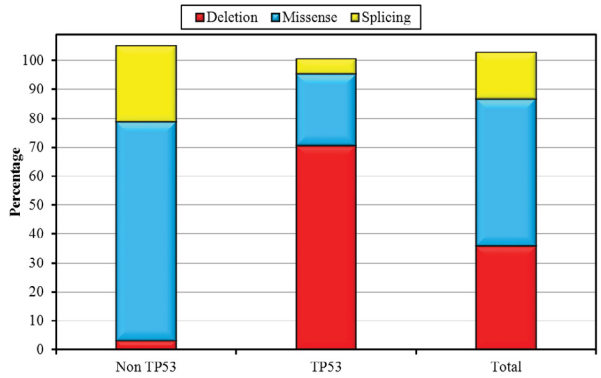

Figure 1: Comparison of variant percentage among TP53 and the other studied genes.

Patients showed different response to anti-EGFR target therapy

Finally, we tried to correlate the identified variants with target therapy. Not all patients were subjected to anti-EGFR target therapy since this therapy should be covered by patient's insurance. Patients IDs 4, 8, 12, 17 and 24 were subjected to avastin. Patients IDs 4, 17 and 24 were good responders with stable disease. Patient ID 4 has got *TP53-KRAS- PIK3CA* pathogenic variants: *TP53* mutation was G>A [rs121912651, Arg248Trp] in exon 7, *KRAS* pathogenic variant was C>T rs121913529 Gly12Asp in exon 2. The latter mutation is one of *KRAS* mutations that predict anti-EGFR response [36] which appeared in our study to be sensitive to avastin with stability of the disease after applying the drug. The *PIK3CA* pathogenic variant was G>A rs104886003 Glu545Lys. For the second good responder; patient ID 17 she has got two pathogenic variants in *TP53*; G>A rs587782529 Arg337Cys in exon 10 and C>T rs28934578 Arg175His in exon 5. However, patient ID 24 did not have any pathogenic variant and the identified variants in this patient were benign, tolerated by SIFT and neutral by Provean predictors. Nevertheless, patients ID 8 and 12 showed no response and died. For patient ID 8, two pathogenic variants were found; *TP53* C>A, rs121912654 Val157Phe in exon 5 and *KRAS* C>T rs121913530 Gly12Ser in exon 2. However, for patient ID 12 no pathogenic variant was detected only a homozygous TT rs41736 was found in exon 20 in *MET* gene; This germline mutation was reported in CRC and siblings with 18% allelic frequency [37]. In brief, most of CRC patients who have different pathogenic variants showed variable response to anti-EGFR therapy.

Discussion

Basically, CRC is a disease that results from an interplay between

common and rare variants with different penetrance [38]. This genetic predisposition will assist in screening high-risk family for CRC. Multiple genetic pathways are involved in the pathogenesis of CRC e.g. *WNT* signalling pathway (adenomatous polyposis coli gene (APC), BubR1), *RAS* pathway (*KRAS*, *BRAF*, mitogen-activated protein kinase *MAPK*), *P53* pathway with the involved genes: *PI3KCA* [39]. With the introducing of advanced molecular techniques e.g. next-generation sequencing multiple genetic defects were investigated through genome-wide studies [40-42]. In the present study, we were able to investigate 15 genes generally affected in CRC through TruSight sequencing panel (Illumina). We found 16 pathogenic variants in 12 patients (50% of total patients). Six of the patients participating in this study had *TP53* missense pathogenic variants (25%) which are comparable to other population (33%) [43]. However, no one of those six patients met classical Li-Fraumni syndrome [44] or Li-Fraumeni-like criteria [45]. It was reported that *TP53* germline mutations were found in 4-5% in Li-Fraumni syndrome (LFS). However, presence of *TP53* mutations with absence of personal and family history that met LFS criteria was also observed in previous studies [46,47]. Consequently, *TP53* had the highest number of investigated mutations including missense, short deletions and splicing mutations as mentioned in results sections; I. a, II and III. Most of them were germline, meaning potentially early onset of the incidence of CRC. Therefore, it is highly recommended to families of those patients to be subjected to direct DNA sequencing of *TP53* exons 2-11 as a screening tool [48]. Additionally, eleven patients were found to have *KRAS* mutations (~ 46%), which is matching with the published percentage (30% to 50%) [49,50]. The importance of *KRAS* mutations are its impact on anti-EGFR target therapy in metastatic colon cancer, with better overall survival for patients carry wild *KRAS* mutation than mutant *KRAS* [51]. Downregulation of *KRAS* subsequenct overexpression of EGFR inhibits PI3K-ERK signalling pathways. Patient ID 4 carried one of the common wild *KRAS* mutation in codon12 (G>A, Gly12Asp, G12D, rs121913529) [52]. This patient showed good response to anti EGFR target therapy. That codon 12 *KRAS* mutation was also detected in patient ID 11. Another common codon 12 *KRAS* wild mutation (Gly12Ser) was found in patient ID 8 with progression and subsequent death in spite of pantimumab target therapy. Codon 13 wild *KRAS* common mutation (Gly13Asp) was detected in patient's ID 5 and 16. Unfortunately, both patients were not subjected to anti-EGFR therapy in order to follow the drug response. Interestingly, patient ID 20 had a wild *KRAS* mutation outside the hotspot codons (Ala146Thr); this mutation confers phenotype similar to mutations in the hotspot codons thus a similar response to chemotherapy [53]. Another modulator of *MAPK* pathway is *BRAF* gene. The coexistence of both *KRAS* and *BRAF* is uncommon in CRC patients. In the present study, patients ID 3 and 19 carried 2 different *BRAF* mutations: Val600Glu (A>T, rs113488022) [54] and Gly596Val (C>A), both variants are pathogenic with different molecular, pathological characteristics and clinical outcomes [55]. These two patients could be benefit from anti-BRAF in combination with anti-EGFR or anti-MAPK [56]. Overall, *BRAF* mutations (mutant or wild) have worse overall survival than *KRAS* mutation carriers. Finally, *PIK3CA,* which plays important role in *MAPK* pathway, has got pathogenic missense variants in patient ID 4 (G>A, rs104886003, Glu545Lys) and patient ID 16 (A>G, rs121913279, His1047Arg,). Both variants are *PIK3CA* hotspot somatic mutations [57] with good response to cetuximab [25]. Interestingly, patient ID 4 is a very good example of a possible interplay between pathways of *TP53*, *KRAS* and *PI3K*. These patients have 3 pathogenic variants; one in each previous gene and showed a good respond to anti-EGFR therapy. However, patient ID 8, who had also 2 pathogenic variants in *P53* and *KRAS*, was bad responder and died. This likely correlation between previous variants could be based on potential molecular cross-talk between different pathways since activation of *AKT* inhibits *TP53*-mediated apoptosis. In addition, mutant *KRAS* antagonizes the downstream of *PI3K* signalling in colon cancer cell lines [58]. Remarkably, thirteen patients (54% of patients) included in the present study had 11 *ERBB2* missense mutations with amino acid substitutions; 8 of them were predicted as damaging disease-causing variants. These variants may be associated with *ERBB2* protein overexpression, thus potential response to anti-Her2/neu (trastuzumab) in combination with other agents e.g. cetuximab and pertuzumab [59,60].

Conclusion

To our knowledge, this is the first CRC study performed in Egypt using Trusight colorectal cancer panel. Although the relative small number of patients involved, this study is considered a good start to know the potential variants found in Egypt and the affected genes to be more focused and investigated.

Acknowledgment

Acknowledgment is to Genetic Signature Cancer Center, Tanta University, Egypt

Funding

This work was funded by grants from Tanta University and Ministry of Higher Education, Egypt

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