Inhibition of MEIS3 Generates Cetuximab Resistance through c-Met and Akt

Ping Cai,1,2,3 Yangyang Xie,2,3,4 Mingjun Dong,1,2,3 and Qiaoqiao Zhu1,2,3

1Anorectal Surgery, Hwa Mei Hospital, University of Chinese Academy of Sciences, 41 Northwest Street Road, Haishu District, Ningbo 315800, China
2Ningbo Institute of Life and Health Industry, University of Chinese Academy of Sciences, 41 Northwest Street Road, Haishu District, Ningbo 315800, China
3Key Laboratory of Diagnosis and Treatment of Digestive System Tumors of Zhejiang Province, 41 Northwest Street Road, Haishu District, Ningbo 315800, China
4Department of Medical Experiment, Hwa Mei Hospital, University of Chinese Academy of Sciences, 41 Northwest Street Road, Haishu District, Ningbo 315800, China

Correspondence should be addressed to Qiaoqiao Zhu; zqqsssemail@163.com

Received 27 June 2020; Revised 12 November 2020; Accepted 25 November 2020; Published 9 December 2020

1. Introduction

In China, the number of newly diagnosed colorectal cancer cases reached 432,000, making colorectal cancer one of the most commonly diagnosed cancers [1]. In stage I patients, the 5-year survival rate is approximately 90%; however, when metastasis occurs, the survival rate drops to 14% [2]. To prolong the survival rate of the patients, multiple studies have attempted to clarify the critical pathway in the proliferation and metastasis of colon cancer. Currently, the target pathways that have been discovered include vascular endothelial growth factor (VEGF) [3], VEGFR [4], EGFR [5], and B-Raf (BRAF) [6].

Cetuximab is an inhibitor of EGFR, which is used to treat metastatic colon cancer, non-small-cell lung cancer, and head and neck cancer [7]. In July 2009, cetuximab was approved by the Food and Drug Administration (FDA) for the treatment of colon cancer with wild-type Kirsten ras sarcoma viral oncogene (KRAS) [8]. Thirty-one clinical trials have thus far been conducted for the treatment of metastatic colon cancer patients including cetuximab as monotherapy or in combination with chemotherapy [9]. Concurrently, the drug resistance to cetuximab has also increased; the mechanism of cetuximab resistance involves mutation in the KRAS gene [10], activation of upstream genes such as C-MET [11], and upregulation of the downstream genes such as AKT [12]. In this study, we attempted to use a high-throughput screening approach to locate genes that are responsible for cetuximab resistance. The MEIS3 gene is a relatively new gene, which encodes a homeobox domain, so it can bind to DNA and regulate the binding of specific DNA sequences, promoter transcription activity, and so on.
In mice, MEIS3 gene expression can regulate the expression of protein 3-phosphatidylinositol-dependent protein kinase-1 [14]. In the mesoderm stage, the change of the MEIS3 expression level can significantly affect the activity of the Wnt signaling pathway and determine the fate of cells. However, the relationship between the MEIS3 gene and cetuximab resistance has not been reported [15].

2. Materials and Methods

2.1. In Silico Analysis. The in silico analysis in this study was completed using the R 3.5.1 software. The cetuximab-sensitive genes were identified in the following manner. (1) We first extracted the drug screen data from the Sanger Institute (https://www.cancerrxgene.org/), and among these datasets, 43 colon cancer cell lines were selected and ranked by the half-maximal inhibitory concentration (IC50) of cetuximab. (2) The top three and the lowest three cell lines were selected, and the RNA-seq data of these six cell lines were extracted. (3) The RNA-seq reads were first normalized by log, transformation, and the differentially expressed genes between IC50 high and low cell lines were calculated using the limma package (linear models for microarray data); 933 genes with FC (fold change) < 2 and P value < 0.05 were identified as downregulated genes. (4) The sgRNA synthesis and library readout were completed by the BGI Group (Beijing Genomics Institute, China). (5) Regarding The Cancer Genome Atlas (TCGA) database, the RNA-seq and clinical data from TCGA-Colon Adenocarcinoma (TCGA-COAD) Genome Atlas (TCGA) database, the RNA-seq and clinical data provided by the manufacturer. The in silico analysis in this study was performed using the limma package (linear models for microarray data); 933 differentially expressed genes between IC50 high and low cell lines were calculated using the limma package (linear models for microarray data); 933 genes with FC (fold change) < 2 and P value < 0.05 were identified as downregulated genes. (4) The sgRNA synthesis and library readout were completed by the BGI Group (Beijing Genomics Institute, China). (5) Regarding The Cancer Genome Atlas (TCGA) database, the RNA-seq and clinical data from TCGA-Colon Adenocarcinoma (TCGA-COAD) dataset were first downloaded using the GDCRNATools package, and then, the relationship between the expression of MEIS3 and patients’ overall survival rate was detected using the survival package.

2.2. Patient Samples. Seventeen cetuximab-sensitive and nine cetuximab-resistant tumor samples were obtained from the Ningbo Beilun People’s Hospital. All the patients included in this study signed an informed consent, and the ethics committee of the Ningbo Beilun People’s Hospital approved all the procedures, and the approval number is YJ-KYSB-NBEY-2018-028-01.

2.3. Cell Culture and Transfection. Colon cancer cell lines CaR-1, CCK-81, SUN-61, CL-40, LOVO, HT-29, KM-12, CL-11, LS-180, LS-513, and MDS-T8 were purchased from ATCC company, and all the cell lines above were cultured in Dulbecco’s modified Eagle medium (DMEM)+10% fetal bovine serum (FBS) medium. The cultures were maintained in Dulbecco’s modified Eagle medium (DMEM)+10% fetal bovine serum (FBS) medium. The cultures were maintained at 37°C with 5% CO2. shRNA and cDNA used in this experiment were synthesized by BGI (Beijing Genomics Institute) company. The transfection of vector was performed using Lipofectamine 2000 reagent (Thermo Fisher Scientific, US); in the transfection, Lipofectamine reagent and vector were first diluted by Opti-MEM medium and then mixed together and cultured for 10 min in room temperature; DNA-lipid complex was then added to cells, and the transfection procedure was finished. And for the cell line experiment, LOVO cells were divided into the LOVO-CON and LOVO MEIS3 shRNA groups, then transfected with CON and MEIS3-shRNA vectors for 48 h, and served for subsequent experiment. In the meantime, SNU-61 cell lines were divided into the CON and MEIS3 groups and transfected with CON and MEIS3 overexpression vectors for 48 h.

2.4. Quantitative Reverse Transcription PCR (qRT-PCR)

2.4.1. mRNA Extraction. First, the tumor tissues from cetuximab-resistant and cetuximab-sensitive patients were homogenized and dissolved in TRIZol reagent. The solution was then mixed with chloroform to separate the mRNA, which was then precipitated using isopropanol and washed with 75% ethanol.

2.4.2. Reverse Transcription. mRNA was then reverse transcribed using the PrimeScript RT Reagent Kit with gDNA Eraser (RR047B, Takara Company, Dalian, China). The transcription process was performed according to the instructions provided by the manufacturer.

2.4.3. qPCR. After cDNA was obtained using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the control, the relative expression of MEIS3 was quantified using the TB Green Advantage qPCR premixes (639376, Takara Company, Dalian, China). The quantification method used was 2ΔΔCt, and the primer sequence was GAPDH F: 5′-GCTTCTGTGACTTCAACAGCG-3′ and R: 5′-ACACACCTTGTTGTGTAAGC-3′ and MEIS3 F: 5′-ATCATATGCGGCCCTTGTTGTCCTC-3′ and R: 5′-CATAGGTGCACGA TGCGTCTC-3′.

2.5. Western Blot. The western blot was performed in the following manner. (1) The cells were first dissolved in radioimmunoprecipitation assay (RIPA) buffer on ice for 30 min; the loading buffer was then added and boiled for 15 min. (2) The sample was loaded onto the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to separate the protein by molecular weight. (3) The proteins were then transferred to polyvinylidine fluoride (PVDF) membranes and blocked with 5% bovine serum albumin (BSA) for two hours. (4) The membranes were labeled with the first antibody, which included the following: GAPDH (Cat No. 5174), Akt (Cat No. 4691), p-Akt (Cat No. 4060), c-Met (Cat No. 8198), phospho-c-Met (Cat No. 3077), EGFR (Cat No. 2085), and p-EGFR (Cat No. 2244), which were all purchased from Cell Signaling Technology (MA, USA). MEIS3 (Cat No. PA5-61288) antibody was purchased from Thermo Fisher Scientific (Waltham, MA, USA). (5) The membranes were labeled with horseradish peroxidase (HRP) secondary antibodies, which were all purchased from Zhongshan Jinqiao Biotechnology Co., Ltd. (Beijing, China), and finally, the protein expression was detected using SuperSignal HRP Chemiluminescent Substrates (Thermo Fisher Scientific, MA, USA).

2.6. Drug Treatment. For this study, cetuximab (A2000), crizotinib (S1068), and miltefosine (S3056) were all purchased from Selleck Chemicals Llc (Houston, Texas, USA). The concentration of drugs that were used for treatment is described...
Figure 1: Continued.
2.7. Cell Proliferation, Apoptosis, and Cell Cycle

2.7.1. Cell Proliferation. The cells were first plated on a 24-well plate and were treated with the MTT reagent subsequent to cell attachment. The plate was then washed twice with phosphate-buffered saline (PBS), 0.1% MTT was added, and the cells were cultured at 37°C for 20 min. The plate was then washed with PBS, and isopropanol was added. Finally, cell proliferation was determined at optical density (OD) 450 nm.

2.7.2. Apoptosis. After the cetuximab treatment, the cells were harvested and washed twice with PBS, then suspended in binding buffer, and labeled with Annexin V APC antibody (Sanjian Company, Tianjin, China). Prior to detection using the fluorescence-activated cell sorting (FACS) technique, the cells were labeled with propidium iodide (PI) to identify the dead cells.

2.7.3. Cell Cycle. After cetuximab treatment, the cells were harvested, washed with PBS, and were fixed in 70% ethanol overnight. The fixed cells were treated with RNase and labeled with a high concentration of PI for 20 min. Finally, the cell cycle was detected by FACS.

3. Results

3.1. Identification of MEIS3 as a Cetuximab-Sensitive Gene. In this study, we first extracted drug-sensitive information from the Sanger Institute. Forty-three colon cancer cell lines with different IC50 values were identified in the cetuximab treatment dataset. After their ranking by the IC50 data, we chose the top three cell lines as cetuximab-resistant cell lines and the last three cell lines as cetuximab-sensitive cell lines (Figure 1(a)). Using limma to analyze the transcriptional data, we identified 933 genes that were downregulated in cetuximab-resistant cell lines (Figure 1(b)). Then, the 933 genes were assigned to 2799 sgRNAs and transfected into CaR-1, CL40, and KM-12 cells. Cells were selected with cetuximab, and next-generation sequencing (NGS) was used to readout the library representation. We confirmed that sgRNA targeting MEIS3 had the highest enrichment ratio among the 2799 sgRNAs (Figures 1(c) and 1(d)). Using our in-house data, we detected the expression of MEIS3 in 17 cetuximab-sensitive and nine cetuximab-resistant patients and confirmed that MEIS3 had a low expression in all the cetuximab-resistant samples at the mRNA level (Figure 1(e)). Further, MEIS3 had a low expression in six cetuximab-resistant cell lines was extracted, and the expression of MEIS3 was detected by western blot (n = 3). (g) The relationship between the expression of MEIS3 and the overall survival rate of COAD patients in TCGA database.

![Figure 1](image-url)

**Figure 1:** (a) Based on the IC50 of cetuximab in 43 colon cancer cell lines from the Genomics of Drug Sensitivity in Cancer (GDSC) dataset, we selected SNU-C1, CL-34, and SW1417 as cetuximab-resistant cell lines and HT-155, HCC2998, and DiFi-09844799147 as cetuximab-sensitive cell lines. (b) The RNA expression profile in both cetuximab-sensitive and cetuximab-resistant cells was obtained, and genes with low expression in cetuximab-resistant cell lines were identified. (c) Pipeline of transfection of sgRNA library and drug selection. (d) NGS readout was used to quantify sgRNA. (e) mRNA from bulk tumor tissue was extracted from 17 cetuximab-sensitive and nine cetuximab-resistant patients, and expression of MEIS3 was identified by qPCR. (f) Bulk tumor tissue protein from eight cetuximab-sensitive and six cetuximab-resistant cell lines was extracted, and the expression of MEIS3 was detected by western blot (n = 3). (g) The relationship between the expression of MEIS3 and the overall survival rate of COAD patients in TCGA database.

in Results. The drug library was designed and completed by Prof. Xingguo Zhang.
Relative expression

(a) MEIS3 expression

(b) CaR-1
CCK-81
SNU-61
CL-40
VoVo
HT-29
KM-12
CL-11
LS-180
LS-313
MDS8

(c) G1=51% S=31% G2=18%
G1=57% S=26% G2=17%
G1=67% S=20% G2=13%
G1=69% S=17% G2=14%
G1=74% S=13% G2=13%
G1=77% S=15% G2=13%
G1=60% S=26% G2=13%
G1=53% S=34% G2=13%
G1=56% S=35% G2=12% G2=13%

(d) G1=43% S=30% G2=27%
G1=43% S=35% G2=22%
G1=53% S=25% G2=17%
G1=56% S=34% G2=8%
G1=59% S=30% G2=11%
G1=67% S=25% G2=8%
G1=70% S=19% G2=11%
G1=72% S=18% G2=10%
G1=77% S=15% G2=7%

Figure 2: Continued.
cancer patients (Figure 1(g)). Considering these results, we confirmed that MEIS3 is a cetuximab-sensitive gene.

3.2. Manipulation of Cetuximab Sensitivity by MEIS3. To further clarify the role of MEIS3 in cetuximab sensitivity, we analyzed 11 colon cancer cell lines in our lab; QPCR confirmed that SNU-61 had the lowest expression of MEIS3, while LOVO had the highest expression (Figure 2(a)). We hence designed MEIS3 shRNA and transfected into LOVO cells considering that MEIS3 shRNA could knockdown MEIS3 protein level (Figure 2(b)); LOVO-CON and LOVO MEIS3 short hairpin RNA (shRNA) were treated with different doses of cetuximab for 48 h; as the concentration of cetuximab was increased in the CON group, the G1 ratio of LOVO cells increased, while in the MEIS3 shRNA cells, the increase in the G1 ratio was slower than that in the CON group, which indicated that in MEIS3-inhibited cells, the G1 arrest induced by cetuximab is alleviated (Figure 2(c)).

In the MTT assay, after treatment with 0.5 μg/ml and 1 μg/ml cetuximab, the survival rate of the MEIS3 shRNA group was significantly higher than that of the CON group (Figure 2(d)). Using SNU-61, which is another MEIS3 low-expression cell line, we further confirmed that overexpression of MEIS3 not only promoted G1 arrest induced by cetuximab (Figures 2(e) and 2(f)) but also inhibited the growth of SNU-61 cells (Figure 2(g)). Furthermore, after treatment with cetuximab, apoptosis of LOVO MEIS3 shRNA was significantly lower than that of LOVO, and SNU-61 MEIS3 was significantly higher than that of SNU-61 CON (Figure 2(h)). Thus, the expression of MEIS3 could determine cetuximab sensitivity.

3.3. Inhibition of p-EGFR Was Delayed When MEIS3 Was Downregulated. Since the mechanism of action of cetuximab is the inhibition of EGFR phosphorylation, we investigated the changes in EGFR phosphorylation. As previously described, when LOVO-CON and MEIS3 shRNA were treated with different concentrations of cetuximab, EGFR phosphorylation in the LOVO-CON group decreased dramatically after treatment with 0.125 μg/ml cetuximab; however, EGFR phosphorylation in the MEIS3 shRNA group did not change until treatment with 1 μg/ml cetuximab, which is further indicated by gray intensity ratio (gray intensity ratio in p-EGFR/gapdh ratio); in LOVO-CON, gray intensity ratio in p-EGFR was 0.29, 0.10, 0.05, 0.03, 0.02, and 0.001, and in LOVO-MEIS3 shRNA, it is 1.52, 1.63, 1.32, 1.18, 1.13, and 0.75 (Figures 3(a) and 3(b)). When MEIS3 cDNA was transfected into the SNU-61 cell line, the phosphorylation of EGFR in the CON group was inhibited after treatment with 1 μg/ml cetuximab, while in the MEIS3 group the phosphorylation of EGFR was inhibited after treatment with 0.25 μg/ml cetuximab (Figures 3(c) and 3(d)). These results indicate that the expression of MEIS3 correlated with the phosphorylation of EGFR. When MEIS3 was inhibited in LOVO cells, the phosphorylation of EGFR increased while the total EGFR was unchanged (Figure 3(e)). Likewise, when MEIS3 was overexpressed, phosphorylation of EGFR was decreased (Figure 3(f)). Based on these results, we concluded that MEIS3 could control the phosphorylation of EGFR.

3.4. Using Drug Library to Overcome Cetuximab Resistance. Considering that the inhibition of MEIS3 could introduce cetuximab resistance, we used high-throughput approaches to overcome this resistance; a drug library containing 146 drugs was applied, and each drug was assigned at five concentrations. By calculating IC50, we found that LOVO-CON, LOVO MEIS3 shRNA, SNU-61 CON, and SNU-61
MEIS3 were all sensitive to inhibitors of c-Met such as crizotinib and XI-184 as well as to miltefosine, which is an inhibitor of Akt (Figure 4(a)). To validate our theory, we again treated the cells in 24-well plates, which further confirmed that LOVO-CON, LOVO MEIS3 shRNA, SNU-61 CON, and SNU-61 MEIS3 were all sensitive to both crizotinib (Figure 4(b)) and miltefosine (Figure 4(d)). Consistent with this result, the phosphorylation of c-Met was decreased after crizotinib treatment (Figure 4(c)), and phosphorylation of Akt additionally decreased after treatment with miltefosine (Figure 4(e)). Furthermore, after MEIS3 is inhibited in LOVO cell lines, p-c-Met and p-Akt are upregulated as expected; however, the elevated p-c-Met and p-Akt have still been inhibited by crizotinib and miltefosine treatment, suggesting that crizotinib and miltefosine served as powerful inhibitors of c-Met and Akt (Figure 4(f)). Concurrently, MEIS3 was unchanged during both drug treatments; considering that the c-Met pathway could regulate the Akt pathway, we suspected that MEIS3 might locate on the upstream of Akt and c-Met.

3.5. Regulation of Phosphorylation of c-Met and Degradation of Akt by MEIS3. Both crizotinib and miltefosine could overcome the cetuximab resistance caused by MEIS3 inhibition; however, these two drugs could not change the expression of MEIS3. This indicated that these 2 drugs might be concentrated on MEIS3 downstream pathways; given that crizotinib and miltefosine are targeting c-Met and Akt, we suspected that MEIS3 might be able to regulate the c-Met and Akt pathways. Therefore, we constructed LOVO-CON, MEIS3 shRNA, MEIS3 shRNA+c-Met OE (overexpression), MEIS3 shRNA+c-Met shRNA, and MEIS3 OE+c-Met shRNA cell
Western blotting indicated that when c-Met was overexpressed, the phosphorylation of EGFR reached the highest level among these five groups, and when c-Met was inhibited, the phosphorylation of EGFR decreased. Concerning Akt, the inhibition of MEIS3 increased the phosphorylation of Akt and total Akt compared with the MEIS3 shRNA + c-Met.
Figure 5: (a) Protein levels of LOVO-CON, MEIS3 shRNA, MEIS3 shRNA+c-Met overexpression, MEIS3 shRNA+c-Met shRNA, and MEIS3 overexpression+c-Met shRNA were extracted, and the expression of p-EGFR, EGFR, p-Akt, Akt, p-c-Met, and c-Met at the protein level was detected by western blot. (b) The LOVO and LOVO MEIS3 shRNA were treated with CHX for different times, and the expression of Akt was detected by western blot. (c) The SNU-61 cell line was treated with CHX for different times, and the expression of Akt was detected by western blot. (d) The LOVO cells were first treated with CHX followed by DMSO, BAF, and MG132 for different times, and the expression of Akt was determined by western blot. (e) SNU-61 (A) and HCT-116 (B) were transfected with CON and MEIS3 shRNA and then treated with DMSO, MG132, and BAF, and the degradation of Akt was determined by western blot. (f) LOVO (A) and SNU-61 (B) were transfected with the CON and MEIS3 vectors, and the ubiquitin-bonded Akt was detected using IP. (g) The LOVO cell line was transfected with random shRNA, CON vector, and c-Met shRNA for 48 h, and then, western blot was used to detect the change of c-Met in the protein level.
OE group thereby suggesting that the expression of Akt could be regulated by MEIS3. Concurrently, the phosphorylation of c-Met was increased after MEIS3 was inhibited, which indicated the MEIS3-c-Met-EGFR axis (Figure 5(a)). As the expression of total Akt is increased after MEIS3 is inhibited, we first treated LOVO-CON and LOVO MEIS3 shRNA with cycloheximide (CHX) and then harvested the protein at different time points. By detecting the change in Akt, we concluded that the degradation of Akt was inhibited after MEIS3 expression was decreased (Figure 5(b)). In the MEIS3 OE cells, degradation of Akt was highly promoted (Figure 5(c)). When treated with the autophagy inhibitor bafilomycin A1 (BAF), the expression of Akt decreased even after CHX treatment. Using the ubiquitination inhibitor MG132, the degradation of Akt was prevented, which indicated that the degradation of Akt was completed by the ubiquitination pathway (Figure 5(d)). Similarly, in SNU-61 and HCT-116 cell lines, transfection of MEIS3 cDNA increased the degradation of Akt, which was stopped by MG132 (Figure 5(e)). Furthermore, using immunoprecipitation (IP) experiments we confirmed that when MEIS3 is inhibited, ubiquitin-bound Akt is decreased (Figure 5(f)). These results show that MEIS3 could regulate the phosphorylation of c-Met; further, ubiquitination of AKT is also regulated by MEIS3.

4. Discussion

Although the application of cetuximab has been confirmed, a phenomenon of cetuximab resistance has been identified. The mechanism of resistance to cetuximab is closely related to its mechanism of action. The known mechanisms include (1) binding to the EGFR receptor on the surface of tumor cells with high affinity and competitively blocking the binding of EGF and EGFR, thus inhibiting the expression of downstream genes; (2) binding to the Fc fragment on the surface of immune cells to induce cell-mediated cytotoxicity; and (3) inducing EGFR into the cell and reducing the phosphorylation level of EGFR, thus inhibiting the proliferation and metastasis of tumor cells [10]. However, most patients will develop drug resistance after 6–12 months of cetuximab treatment; it is thus essential to understand the underlying mechanism to overcome this resistance [16]. The mechanism of cetuximab resistance includes primary and secondary resistance. In primary resistance, patients develop drug resistance during the first treatment. It accounts for 60% of the total incidence of resistance to the drug [17]. Most of the primary resistance was due to the abnormal activation of EGFR downstream or upstream genes such as KRAS [18], PI3K/AKT [19], and IGF1 [20]. Part of the cetuximab resistance was due to compensation by the NF-κB pathway [21]. Most of the secondary resistance was due to mutations in EGFR [22].

In this study, we first identified genes that were downregulated in cell lines that are less sensitive to cetuximab using sgRNA screening. We confirmed that after cetuximab treatment, sgRNA targeting MEIS3 was highly expressed in surviving cells; further proliferation, apoptosis, and cell cycle analysis concluded that the expression of MEIS3 could change cetuximab sensitivity. MEIS3 belongs to the MEIS family of proteins, which forms dimeric or trimeric DNA-binding complexes with PBX and/or HOX proteins [23] and is widely involved in cell functions such as neural stem cell development [24] and hematopoiesis [23]. Unlike MEIS1 and MEIS2, the function of MEIS3 is not well known. To clarify and overcome cetuximab resistance caused by MEIS3 inhibition, we applied a drug library containing 146 drugs. Through drug screening, we identified that the MEIS3 shRNA cell line was sensitive to c-Met and Akt pathway inhibitors. Further analysis indicated that MEIS3 could alter the phosphorylation of EGFR and Akt, and most importantly, MEIS3 could change the ubiquitination of Akt.

5. Conclusion

Our results indicate that MEIS3 functions as a cetuximab-sensitive gene, which works through the phosphorylation of c-Met and degradation of Akt.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The study is supported by the Ningbo Municipal Natural Science Foundation (2019A610331) and Basic Public Welfare Research Project of Zhejiang Province (LGC20H160002).

References

[1] C. J. Kahi, C. R. Boland, J. A. Dominitz et al., “Colonoscopy surveillance after colorectal cancer resection: recommendations of the US multi-society task force on colorectal cancer,” Gastroenterology, vol. 150, no. 3, pp. 758–768.e11, 2016.
[2] L. Lamartina and D. Handkiewicz-Junak, "Follow-up of low risk thyroid cancer patients: can we stop follow-up after 5 years of complete remission?," European Journal of Endocrinology, vol. 182, no. 5, pp. D1–D16, 2020.
[3] S. Lee, H. J. Kim, S. C. Oh, and D. H. Lee, “Genipin inhibits the invasion and migration of colon cancer cells by the suppression of HIF-1α accumulation and VEGF expression,” Food and Chemical Toxicology, vol. 116, Part B, pp. 70–76, 2018.
[4] H. Nagano, C. Tomida, N. Yamagishi, and S. Teshima-Kondo, “VEGFR-1 regulates EGF-R to promote proliferation in colon cancer cells,” International Journal of Molecular Sciences, vol. 20, no. 22, p. 5608, 2019.
[5] P. Luraghi, V. Bigatto, E. Cipriano et al., “A molecularly annotated model of patient-derived colon cancer stem–like cells to assess genetic and nongenetic mechanisms of resistance to anti-EGFR therapy,” Clinical Cancer Research, vol. 24, no. 4, pp. 807–820, 2018.
[6] J. Taieb, K. Le Malicot, Q. Shi et al., “Prognostic value of BRAF and KRAS mutations in MSI and MSS stage III colon cancer,” Journal of the National Cancer Institute, vol. 109, no. 5, 2016.
[7] V. Subbiah, E. I. Dumbrava, Y. Jiang et al., "Dual EGFR blockade with cetuximab and erlotinib combined with anti-VEGF antibody bevacizumab in advanced solid tumors: a phase 1 dose escalation triplet combination trial," *Experimental Hematology & Oncology*, vol. 9, no. 1, pp. 1–8, 2020.

[8] B. A. Weinberg, M. L. Hartley, and M. E. Salem, "Precision medicine in metastatic colorectal cancer: relevant carcinogenic pathways and targets—part 1: biologic therapies targeting the epidermal growth factor receptor and vascular endothelial growth factor," *Oncology*, vol. 31, no. 7, 2017.

[9] K. Mody and T. Bekaii-Saab, "Clinical trials and progress in metastatic colon cancer," *Surgical Oncology Clinics*, vol. 27, no. 2, pp. 349–365, 2018.

[10] H. Kuramochi, A. Nakamura, G. Nakajima et al., "PTEN mRNA expression is less pronounced in left-than right-sided colon cancer: a retrospective observational study," *BMC Cancer*, vol. 16, no. 1, p. 366, 2016.

[11] N.-J. Chiang, C. Hsu, J.-S. Chen et al., "Expression levels of ROS1/ALK/c-MET and therapeutic efficacy of cetuximab plus chemotherapy in advanced biliary tract cancer," *Scientific Reports*, vol. 6, no. 1, 2016.

[12] S. Zhang, Y. Zhang, J. Qu et al., "Exosomes promote cetuximab resistance via the PTEN/Akt pathway in colon cancer cells," *Brazilian Journal of Medical and Biological Research*, vol. 51, no. 1, 2018.

[13] W. Fong and K. K. W. To, "Drug repurposing to overcome resistance to various therapies for colorectal cancer," *Cellular and Molecular Life Sciences*, vol. 76, 2019.

[14] T. Toyotome, H. Takahashi, and K. Kamei, "MEIS3 is repressed in A549 lung epithelial cells by deoxynivalenol and the repression contributes to the deleterious effect," *The Journal of Toxicological Sciences*, vol. 41, no. 1, pp. 25–31, 2016.

[15] J. Liu, Y. Wang, M. J. Birnbaum, and D. A. Stoffers, "Three-amino-acid-loop-extension homeodomain factor Meis3 regulates cell survival via PDK1," *Proceedings of the National Academy of Sciences*, vol. 107, no. 47, pp. 20494–20499, 2010.

[16] Y. M. Elkouby, S. Elias, E. S. Casey et al., "Mesodermal Wnt signaling organizes the neural plate via Meis3," *Development*, vol. 137, no. 9, pp. 1531–1541, 2010.

[17] M. Gegia, N. Winters, A. Benedetti, D. van Soolingen, and D. Menzies, "Treatment of isoniazid-resistant tuberculosis with first-line drugs: a systematic review and meta-analysis," *The Lancet Infectious Diseases*, vol. 17, no. 2, pp. 223–234, 2017.

[18] L. Trusolino and S. M. Leto, "Resistance of colorectal tumors to anti-EGFR antibodies," in *Resistance to Anti-Cancer Therapeutics Targeting Receptor Tyrosine Kinases and Downstream Pathways*, pp. 1–27, Springer, 2018.

[19] L. Gao, J. Xu, G. He et al., "CCR7 high expression leads to cetuximab resistance by cross-talking with EGFR pathway in PI3K/AKT signals in colorectal cancer," *American Journal of Cancer Research*, vol. 9, no. 11, pp. 2531–2543, 2019.

[20] R. Ferrarotto, W. N. William Jr., J. E. Tseng et al., "Randomized phase II trial of cixutumumab alone or with cetuximab for refractory recurrent/metastatic head and neck squamous cell carcinoma," *Oral Oncology*, vol. 82, pp. 83–90, 2018.

[21] J. E. Jang, H.-P. Kim, S. W. Han, and T. Y. Kim, "Abstract B100: mutant KRAS-induced macrophage migration inhibitory factor (MIF) secretion promotes resistance to cetuximab in colorectal cancer," in *Target Identification and Validation*, 2018.

[22] A. Bagchi, J. N. Haidar, S. W. Eastman et al., "Molecular basis for necitumumab inhibition of EGFR variants associated with acquired cetuximab resistance," *Molecular Cancer Therapeutics*, vol. 17, no. 2, pp. 521–531, 2018.

[23] R. H. Dowen, "CEH-60/PBX and UNC-62/MEIS coordinate a metabolic switch that supports reproduction in C. elegans," *Developmental Cell*, vol. 49, no. 2, pp. 235–250.e7, 2019.

[24] F. Langellotto, M. Fiorentino, E. De Felice et al., "Expression of Meis and hoxa11 in dipnoan and teleost fins provides new insights into the evolution of vertebrate appendages," *Evo-Devo*, vol. 9, no. 1, p. 11, 2018.