Frequency of I655V SNP of HER-2/*neu* in colorectal cancer: a study from India

Rameez Hasan^{1,2} · Deepti Bhatt¹ · Shahbaz Khan¹ · Vasiuddin Khan¹ · Amit Kumar Verma¹ · Prahalad Singh Bharti³ · Afzal Anees² · Kapil Dev¹

Received: 16 September 2018 / Accepted: 16 December 2018 / Published online: 2 January 2019
© King Abdulaziz City for Science and Technology 2019

Abstract

The present study was conducted to determine the prognostic significance of I655V SNP (rs1136201) is a genetic one in HER-2 oncoprotein in cases of colorectal cancer (CRC). We conducted a case–control study analysing 83 subjects (naïve primary CRC cases) who underwent CRC biopsy/colectomy and included 57 healthy control subjects. Analysis of HER-2 polymorphism was done by PCR-RFLP technique. The mean age was found to be 55.9 years; median age was 56 years and mode age was 54 years with a range of 43 (30–73). Males constitute 63 (75.9%) and females constitute 20 (24.1%) of patient population. According to gradewise distribution, 12 (14.45%) patients were of Grade I, 53 (63.85%) of Grade II, and 18 (21.68%) were of Grade III. We found out that out of 83 patients, 52 (62.65%) were of homozygous wild type (A/A; Ile/Ile); 27 (32.53%) were of heterozygous type (A/G; Ile/Val) and 4 (4.81%) were of homozygous mutant type (G/G; Val/Val). Allelic frequency of Ile (A) was found out to be 0.79 and that of Val (G) is 0.21 and were not significantly different from the healthy control population. Fischer's exact *p* value obtained was 0.86.

Keywords HER-2/*neu* polymorphism · I655V (A/G) · Colorectal cancer (CRC) · PCR-RFLP · Prognostic · Biomarker

Abbreviations

CRC	Colorectal cancer
HER	Human epidermal growth factor receptor
JMI	Jamia Millia Islamia
JNMC	Jawaharlal Nehru Medical College
AMU	Aligarh Muslim University
SNP	Single nucleotide polymorphism
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism

ALFRED	Allele frequency database
IDC	Invasive ductal carcinoma

Introduction

Human Epidermal Growth Factor Receptor 2 also known as HER-2/*neu*/ERBB2/CD340 or p185 is a 185 kDa trans-membrane glycoprotein encoded by ERBB2, a known

✉ Kapil Dev
kdev@jmi.ac.in
https://www.jmi.ac.in/biotechnology/faculty-members/Dr_Kapil_Dev-1758

Rameez Hasan
rameezhasan05@hotmail.com

Deepti Bhatt
deeptibhatt.bhatt@gmail.com

Shahbaz Khan
shahbaz.k30@gmail.com

Vasiuddin Khan
vssh.khan@gmail.com

Amit Kumar Verma
amitvrm999@gmail.com

Prahalad Singh Bharti
bharti.pbh@gmail.com

Afzal Anees
afzalanees@gmail.com

¹ Department of Biotechnology, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India

² Department of Surgery, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh 202001, India

³ Department of Biophysics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India

proto-oncogene (Jørgensen 2014), located on chromosome 17q21 and is one of the four members of epidermal growth factor receptor and belongs to the family of membrane tyrosine kinases. Tyrosine kinase activity enables them to play a pivotal role in pathways involving signalling and transduction; dimerization of the HER receptors results into stimulation of HER-2 cell signalling cascades; thus, HER-2 remains to be the ideal heterodimerization ally for all other members of HER family (Wang et al. 2015). HER-2 plays significant roles in growth, survival, and differentiation of the living cell in a multifaceted manner (Wagner et al. 2013). Any mutation or overexpression of HER-2 can result into tumorigenesis (Ng et al. 2015). Interestingly, in rodents, a mutation in HER-2 gene is required for tumorigenesis, while in the human, tumorigenic potential of HER-2 is due to the overexpression of the wild-type HER-2 gene (Tai et al. 2010).

Several drugs and therapeutics have been developed and introduced to target the pathway, and the detection of HER-2 has become a routine pathological procedure in breast cancer, i.e., an established biomarker in breast malignancy (Tan et al. 2011; Iqbal and Iqbal 2014). Moreover, HER-2 is also the prime target of mAb therapies (Dean 2015) or tyrosine kinase inhibitor-based novel therapies against cancer (Singla et al. 2018). However, HER-2 has not yet been accepted as a prognostic marker in case of CRC.

Colorectal cancer is ranked third most common malignancy and fourth according to mortality equally affecting both the genders globally; and it remains a major cause of cancer related deaths in spite of developments in treatment modalities (Ferlay et al. 2014). CRC is leading underlying causal agent of death globally with an incidence of 9% among various cancer types (Arnold et al. 2016). U.S.A, Australia, Canada, New Zealand, and some parts of Europe have higher incidence rates of CRC, while Asian, African, and South American population faces the lowest risk. CRC incidence rate in India is among lowest globally (Patil et al. 2017). At the same time, the present situation is alarming, as various population-based studies are depicting a steep increase in CRC incidence (Meyer and Are 2017). It is a matter of huge concern that the incidence rates are much higher in Indian immigrants to the UK and USA, signifying the role of living manners and food habits in causing CRC (Paszat et al. 2017). It infers that with the fiscal shift of India from developing to the developed economy, there will be much higher CRC cases.

Colorectal cancer survival is strongly correlated with the stage of disease at the time of primary screening and 5-year survival rate varies accordingly from 90% for localized tumors to 70% for regional and up to 10% for distant metastatic cancer (Kolligs 2016). In general, survival chances are maximum at the primitive stage at the initial diagnosis. The chances of CRC diagnosis rises after 40 years of age, gradually increasing from age 40, rising steeply after 50 years

of age (Jung 2017). Prior history of adenomatous polyps is also a major risk factor in the development of CRC (Simon 2016). The progression of malignancy from adenomatous polyps generally needs a long intermittent dormancy period of 5–10 years (Bonnington and Rutter 2016).

At present, accurate prediction of disease outcome is poor and limited as the prognosis and preferred treatment depends only upon the stage of the disease at screening. For the realization of individualised therapy, identification of more and novel disease markers is a must. In several previous studies, researchers tried to unmask association between molecular mechanisms and the causation of CRC. It is generally acknowledged notion worldwide that CRC results from various environmental and hereditary factors, inducing genetic changes in crucial genes involved in cell signalling and growth such as HER-2; alterations in which, such as gene amplification and point mutations, were found to be exist in CRC patients (Marx et al. 2010; Conradi et al. 2013; Sclafani et al. 2013).

I655V SNP (rs1136201) of the HER-2 gene was identified by (Papewalis et al. 1991), which encodes isoleucine (ATC) or valine (GTC) as per the case. Controversies exist in the data of the previous studies regarding prognostic significance of I655V SNP in the progression of CRC, reported from different parts of the world. Though, studying the role of HER-2 in causing CRC in Indian patient population is still an untouched area. Many epidemiological studies examined the association between breast cancer risk and I655V SNP. However, several inconsistencies existed in the results and its role in CRC has been proposed, but no significant study followed. Examining the impact of this frequent HER-2 polymorphism in CRC in Indian patient population is still untouched and can be significant as racial or ethnic differences affect allelic frequency and polymorphism.

Unfortunately, there is not even a single study done in India so far establishing the prognostic role of HER-2 in CRC, nor did any study performed globally included Indian patient population in their samples.

Materials and methodology

Ethical approval and sample collection

The study was done at the Medical Biotechnology Laboratory, Department of Biotechnology, Jamia Millia Islamia, New Delhi after ethical approval of Institutional Ethics Committee (vide Proposal No. 17/9/10/JMI/IEC/2015 dated 14.01.2016) and from collaborating institute (vide D. No. 2183/FM dated 09.02.2016). We conducted a patient based study analysing 83 (Eighty-three) unrelated subjects with histologically verified CRC (naïve primary cases) admitted at Jawaharlal Nehru Medical College, Aligarh Muslim

University, Aligarh who underwent CRC biopsy/colectomy. All participants are Indian and lived in and around the Aligarh district in Uttar Pradesh province of India. All patients were recruited in the study at diagnosis, and the mean age was found to be 55.9 years, median age was 56 years, and mode age was 54 years with a range of 43 (30–73) (Table 1). Males constitute 63 (75.9%) and females constitute 20 (24.1%) of patient population. Clinical parameters were obtained at the diagnosis and 12 (14.45%) patients were of Grade I, 53 (63.85%) of Grade II, and 18 (21.68%) were of Grade III (Table 1). All samples were collected only after obtaining informed consent of the study participants. A tissue biopsy/colectomy sample was collected from a single patient and was stored in 1X PBS, Phosphate Buffer Saline (MP Biomedicals), pH 7.4 at -20°C (Celfrost) and was used for isolation of genomic DNA. We also included 57 healthy control subjects into the study. Venous blood samples were collected from healthy control subjects and used for isolation of DNA.

DNA Isolation

Genomic DNA was isolated using DNA Isolation Kit (DNeasy, Qiagen) as per manufacturer's protocol. DNA isolated was checked for quality and integrity using UV–Vis. Spectrophotometry (Nanodrop 2000, Thermo Fischer) and 0.8% Agarose (Amresco, VWR) Gel Electrophoresis (Peqlab, VWR). DNA yield came out to be 45–50 ng. A_{260}/A_{280} ratio came around 1.8 and intact band without smearing was visible after electrophoresis both of

which indicate that the isolated DNA was of pure quality, free from contamination and intact.

Analysis of HER-2 polymorphism

HER-2 polymorphism (I655V, i.e., A>G) was detected with the use of primers (IDT) as per primer sequences mentioned in (Pinto et al. 2004) (Forward 5'-AGAGCG CCAGCCCTCTGACGTCCAT-3' and Reverse 5'-TCC GTTTCCTGCAGCAGTCTCCGCA-3') in 20 μl reaction mixture volume using GoTaq[®] Green Master Mix (Promega) by the following PCR conditions; 94°C for 5 m, 35 cycles of (94°C —30 s; 62°C —45 s; 72°C —30 s) and 72°C for 7 m in peqSTAR 96 universal gradient thermocycler (Peqlab, VWR). PCR products were checked by 2% Agarose Gel Electrophoresis; Gel images were obtained by Gel Documentation system (Biorad).

HER-2 polymorphism (I655V, i.e., A>G) was detected by digesting 6 μl PCR product (Amplified HER-2) with 2.5 units of restriction enzyme in 10 μl reaction mixture volume; BsmAI (NEB) overnight at 37°C . This restriction enzyme recognizes the sequence GTC; digesting the product to form two fragments of 116 and 32 bp. The wild-type allele (A/A) produced one band of 148 bp; wild-type/variant allele, i.e., heterozygous (A/G), produced three bands of 148, 116, and 32 bp, and the variant allele (G/G) produced two bands of 116 and 32 bp (Fig. 1). The 32 bp band cannot be obtained on Agarose Gel Electrophoresis. Digested PCR products were checked by 3% Agarose Gel Electrophoresis.

Table 1 Clinicopathological characteristics of colorectal cancer patients

Character- istic	Total	Ile(A)/Ile(A)	Val carrier	<i>p</i> value
Gender				
Male	63 (75.9%)	42 (66.66%)	21 (33.33%)	0.196
Female	20 (24.1%)	10 (50%)	10 (50%)	
Grade				
I	12 (14.45%)	3 (25%)	9 (75%)	0.011*
II	53 (63.85%)	35 (66.03%)	18 (33.97%)	
III	18 (21.68%)	14 (77.77%)	4 (22.22%)	
Age				
<45	2 (2.40%)	0	2 (100%)	0.137
≥45	81 (97.6%)	52 (64.19%)	29 (35.8%)	
Range	30–73 (43)	46–73 (27)	30–63(33)	<0.00001*
Mean	55.9	58.38	51.74	
Median	56	58	52	
Mode	54	54, 57	54	

*Significant association at $p < 0.05$

Fig. 1 3% agarose gel electrophoresis for digested PCR product. **M**—50 bp DNA ladder (Promega), (**A/A**)—homo wild; 148 bp, (**A/G**)—hetero; 148 bp, 116 bp and (**G/G**)—homo mutant; 116 bp

Statistical analysis

Fischer exact test was used to compare the observed number of each genotype with the healthy control population and to compare categorical variables, while Mann–Whitney *U* test was used for examining patient age. Significance levels were set at $p < 0.05$.

Results

The frequency of I655V SNP of the HER-2 gene was studied in 83 CRC cases and 57 healthy control subjects by performing standardised PCR–RFLP technique. The genotype and allele frequencies were not significantly different from the healthy control population, as shown in Table 2. There was no statistically significant association between the Val allele with gender (p value 0.196 at $p < 0.05$) as well as age (p value 0.137 at $p < 0.05$), but there was a statistically significant association between the Val allele with grade (p value 0.011 at $p < 0.05$), as shown in Table 1. The distribution of age among both groups is approximately normal (the p value is < 0.00001 and the result is significant at $p < 0.05$), as shown in Table 1.

We found out that out of 83 patients 52 (62.65%) were of Homozygous Wild-type (A/A; Ile/Ile); 27 (32.53%) were of Heterozygous type (A/G; Ile/Val); and 4 (4.81%) were of Homozygous Mutant type (G/G; Val/Val), while for healthy control population 35 (61.40%); 19 (33.33%) and 3 (5.26%) individuals were found to be in respective category, as shown in Table 2. Allelic frequency of Ile (A) was found out to be 0.79 and that of Val (G) is 0.21 for cases, while 0.78 and 0.22 for the healthy controls, respectively, as shown in Table 2. Fischer's exact p value obtained was 0.86 (Table 2). Odds ratio obtained was 0.94 (95% CI 0.47–1.89), as shown in Table 2.

Discussion

We studied I655V SNP of the HER-2 gene in our CRC cases to investigate its prognostic significance in the progression of CRC in India. We found that the observed allele frequency for the Val (G) allele in our patient population is 0.21 which is close to the average Val (G) allele frequency of 0.239 among 59 populations globally according to ALFRED (Allele Frequency Database) database maintained by Yale University, USA accessible from <https://alfred.med.yale.edu/> (Rajeevan et al. 2012) as well as genotype frequencies were found to be nearly identical with that of healthy control population. This shows that the occurrence of I655V SNP is not involved in causation/progression of CRC in Indian patient population, but at the same time, it did not imply any deficit in prognostic worth in another malignancies or normal tissues, particularly in invasive ductal carcinoma (IDC) of breast malignancy. We came across with only a single study involving Scottish Caucasians with Val (G) allele frequency of 0.20 which concluded that this SNP is not related to CRC, i.e., in accordance with our study and supports it (McKay et al. 2002). We did not come across any study in CRC which concluded significant association of this SNP in any population.

Our study was a retrospective study performed on 83 primary colorectal cancer cases. Analysis of I655V SNP polymorphism in HER-2/*neu* showed no significant association between particular type of genotype and colorectal cancer. Genotypes did not show any statistically significant difference between case and healthy control group, thus predicting poor prognostic implication in pathophysiology of colorectal carcinoma. Various genotypes of HER-2 were correlated with their incidence in different grades of tumor, but no statistically significant difference was observed. Therefore, for elucidating exact risk of colorectal malignancy in India, we must identify the putative genes involved in onset and progression of colorectal cancer as well as their genotypes. However, a large cohort study is the need of the hour for molecular analysis. Based

Table 2 HER-2 I655V polymorphism in colorectal cancer cases and healthy controls

Population	No of subjects	No of individuals (%) Genotype frequency			Allelic frequency		Fischer exact p value
		Ile(A)/Ile(A)	Ile(A)/Val(G)	Val(G)/Val(G)	Ile (A)	Val (G)	
Colorectal cancer cases	83	52 (62.65%) 0.63	27 (32.53%) 0.33	4 (4.81%) 0.04	0.79	0.21	0.86
Healthy controls	57	35 (61.40%) 0.61	19 (33.33%) 0.33	3 (5.26%) 0.05	0.78	0.22	
OR			0.94				
95% CI			0.47–1.89				

on these results, we propose that every population should assess its own genomic make-up for risk analysis of cancer which will guide us towards understanding the topographical and ethical alterations reported for incidence and mortality of colorectal malignancy.

Various studies in the past which considered this I655V SNP showed variances in allele frequencies among different racial and ethnic groups (Riaz et al. 2016), which is expected to impact the diagnostic value of this SNP worldwide. There were not any earlier reports on the impact of the I655V SNP on the medical consequences of CRC patients in India. In the present study, no association was found among occurrence of the I655V SNP with the malignancy, demonstrating that assessment of this SNP would be of petite use in CRC. In inference, even though HER-2 appears to be of utmost importance in the causation as well as progression of several other malignancies, evaluation of the I655V SNP would not bring any fruitful outcomes to patients of CRC. Most of the studies done so far in establishing the prognostic and clinical role of HER-2 were focused on Breast Cancer; HER-2 is considered as an established biomarker in breast cancer patients. Very few studies were done so far for CRC, which concluded controversial prognostic role of HER-2 in CRC. Neither of these studies was done in India nor do they include Indian patient population into their studies.

We evaluated prognostic potential of HER-2 in CRC in Indian patient population as the rate of people getting CRC varies by race and ethnicity. Many replicating/linked/derived research studies are done in the past but of no clinical benefit in prevention of CRC at the primary level. If we could be able to identify the factors accountable for the low incidence of CRC in India, it could help in better management of disease at the primary level. In a developing nation as India in which westernization is covering the face of Indian culture and economy is improving year by year, CRC incidences will be going to increase. Clearly, CRC is becoming an important public health problem, not only restricting to western lifestyle countries, but also penetrating its roots in other developing countries. The ageing population globally will surely face the challenges of CRC if effective preventive measures are not taken.

Acknowledgements The authors are indebted to all the study participants. Rameez Hasan would like to thank and acknowledge University Grants Commission (U.G.C.), Govt. of India and Ministry of Minority Affairs, Govt. of India for providing Maulana Azad National Fellowship (F1-17.1/2013-14/MANF-2013-14-MUS-RAJ-20039 dated 6th Feb. 2014) to him.

Author contributions RH and KD planned the study. RH, AA, and KD designed the manuscript. AA performed colonoscopic biopsies/colectomy. RH performed laboratory procedures under the guidance of KD. RH wrote the paper. DB, VK, AKV, PSB, and SK helped in revising the manuscript. All the authors reviewed and approved the manuscript.

Funding This work was funded by the University Grants Commission (U.G.C.), Govt. of India through MRP grant awarded to Kapil Dev (MRP-MAJOR-BIOT-2013-34536) vide F. No. 43-84/2014(SR) dated 23rd Sept. 2015.

Compliance with ethical standards

Availability of data and material We state that research data will not be shared or put in open public domain, as it is forbidden under the policy of JMI and AMU. The data that support the outcomes of this study are accessible from JMI. However, limitations exist in availability of this data, which was used under the authorization for the present study and so is not openly accessible. Data is, however, available from the authors upon reasonable request and with permission of JMI and AMU.

Conflict of interest The authors declare that they have no competing interests.

Ethics approval & consent to participate The Institutional Ethics Committee of Jamia Millia Islamia (vide Proposal No. 17/9/10/JMI/IEC/2015 dated 14.01.2016) and Aligarh Muslim University (vide D. No. 2183/FM dated 09.02.2016) has approved the study. A written informed consent was obtained before inclusion in the study.

References

- Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F (2016) Global patterns and trends in colorectal cancer incidence and mortality. *Gut* 66(4):683–691
- Bonnington SN, Rutter MD (2016) Surveillance of colonic polyps: are we getting it right? *World J Gastroenterol* 22(6):1925
- Conradi LC, Styczen H, Sprenger T, Wolff HA, Rodel C, Nietert M, Homayounfar K, Gaedcke J, Kitz J, Talaulicar R, Becker H, Ghadimi M, Middel P, Beissbarth T, Ruschoff J, Liersch T (2013) Frequency of HER-2 positivity in rectal cancer and prognosis. *Am J Surg Pathol* 37(4):522–531. <https://doi.org/10.1097/PAS.0b013e318272ff4d>
- Dean L (2015) Trastuzumab (herceptin) therapy and ERBB2 (HER2) genotype
- Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Bray F (2014) Globocan 2012, cancer incidence and mortality worldwide: IARC CancerBase No. 11. International Agency for Research on Cancer, Lyon
- Iqbal N, Iqbal N (2014) Human epidermal growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. *Mol Biol Int* 2014:852748. <https://doi.org/10.1155/2014/852748>
- Jørgensen JT (2014) Role of human epidermal growth factor receptor 2 in gastric cancer: biological and pharmacological aspects. *World J Gastroenterol WJG* 20(16):4526
- Jung YS (2017) Is colorectal cancer screening necessary before 50 years of age? *Intest Res* 15(4):550–551
- Kolligs FT (2016) Diagnostics and epidemiology of colorectal cancer. *Visc Med* 32(3):158–164
- Marx AH, Burandt EC, Choschzick M, Simon R, Yekebas E, Kaifi JT, Mirlacher M, Atanackovic D, Bokemeyer C, Fiedler W, Terracciano L, Sauter G, Izbicki JR (2010) Heterogenous high-level HER-2 amplification in a small subset of colorectal cancers. *Hum Pathol* 41(11):1577–1585. <https://doi.org/10.1016/j.humpath.2010.02.018>
- McKay J, Loane J, Ross V, Ameyaw M, Murray G, Cassidy J, McLeod H (2002) c-erbB-2 is not a major factor in the development of colorectal cancer. *Br J Cancer* 86(4):568–573

- Meyer B, Are C (2017) Current status and future directions in colorectal cancer. Springer, India
- Ng CK, Martelotto LG, Gauthier A, Wen H-C, Piscuoglio S, Lim RS, Cowell CF, Wilkerson PM, Wai P, Rodrigues DN (2015) Intratumor genetic heterogeneity and alternative driver genetic alterations in breast cancers with heterogeneous HER2 gene amplification. *Genome Biol* 16(1):107
- Papewalis J, Nikitin AY, Rajewsky M (1991) G to A polymorphism at amino acid codon 655 of the human erbB-2/HER2 gene. *Nucleic Acids Res* 19(19):5452–5452
- Paszat L, Sutradhar R, Liu Y, Baxter NN, Tinmouth J, Rabeneck L (2017) Risk of colorectal cancer among immigrants to Ontario, Canada. *BMC Gastroenterol* 17(1):85
- Patil PS, Saklani A, Gambhire P, Mehta S, Engineer R, De'Souza A, Chopra S, Bal M (2017) Colorectal cancer in India: an audit from a tertiary center in a low prevalence area. *Indian J Surg Oncol* 8(4):484–490
- Pinto D, Vasconcelos A, Costa S, Pereira D, Rodrigues H, Lopes C, Medeiros R (2004) HER2 polymorphism and breast cancer risk in Portugal. *Eur J Cancer Prev* 13(3):177–181
- Rajeevan H, Soundararajan U, Kidd JR, Pakstis AJ, Kidd KK (2012) ALFRED: an allele frequency resource for research and teaching. *Nucleic Acids Res* 40(Database issue):D1010–D1015. <https://doi.org/10.1093/nar/gkr924>
- Riaz SK, Rashid MM, Kayani MA, Arshad Malik MF (2016) Role of HER-2 Ile655Val polymorphism as universal cancer susceptibility marker among different cancers. *Arch Iran Med (AIM)* 19(6):430–438
- Sclafani F, Roy A, Cunningham D, Wotherspoon A, Peckitt C, Gonzalez de Castro D, Tabernero J, Glimelius B, Cervantes A, Eltahir Z, Oates J, Chau I (2013) HER2 in high-risk rectal cancer patients treated in EXPERT-C, a randomized phase II trial of neoadjuvant capecitabine and oxaliplatin (CAPOX) and chemoradiotherapy (CRT) with or without cetuximab. *Ann Oncol* 24(12):3123–3128. <https://doi.org/10.1093/annonc/mdt408>
- Simon K (2016) Colorectal cancer development and advances in screening. *Clin Interv Aging* 11:967
- Singla H, Munshi A, Banipal RP, Kumar V (2018) Recent updates on the therapeutic potential of HER2 tyrosine kinase inhibitors for the treatment of breast cancer. *Curr Cancer Drug Targets* 18(4):306–327
- Tai W, Mahato R, Cheng K (2010) The role of HER2 in cancer therapy and targeted drug delivery. *J Control Release* 146(3):264–275
- Tan LD, Xu YY, Yu Y, Li XQ, Chen Y, Feng YM (2011) Serum HER2 level measured by dot blot: a valid and inexpensive assay for monitoring breast cancer progression. *PLoS One* 6(4):e18764. <https://doi.org/10.1371/journal.pone.0018764>
- Wagner MJ, Stacey MM, Liu BA, Pawson T (2013) Molecular mechanisms of SH2-and PTB-domain-containing proteins in receptor tyrosine kinase signaling. *Cold Spring Harbor Perspect Biol* 5(12):a008987
- Wang DD, Ma L, Wong MP, Lee VH, Yan H (2015) Contribution of EGFR and ErbB-3 heterodimerization to the EGFR mutation-induced gefitinib-and erlotinib-resistance in non-small-cell lung carcinoma treatments. *PLoS One* 10(5):e0128360