List of ten best papers of the candidate

No	Names of the author(s)	Year	Title of the paper	Name of journal	Volume and page	Impact Factor	Citations (Scopus)
1.	Bapat SA, Mali AM, Koppikar CB, Kurrey NK.	2005	Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer.	Cancer Research	65:3025-3029.	8.619	561
2.	Kurrey NK, Kumar A, Bapat SA.	2005	Snail and Slug are major determinants of ovarian cancer invasiveness at the transcription level.	Gynecologic Oncology	97:155-65.	2.919	218
3.	Kurrey NK, Jalgaonkar SP, Joglekar AV, Ghanate AD, Chaskar PD, Doiphode RY, Bapat SA.	2009	Snail and Slug mediate radio- and chemo-resistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells.	Stem Cells	27(9): 2059-2068.	5.614	485
4.	Gardi NL, Deshpande TU, Kamble SC, Budhe SR, Bapat SA.	2014	Discrete molecular classes of ovarian cancer suggestive of unique mechanisms of transformation and metastases.	Clinical Cancer Research	20:87-99.	8.911	24
5.	Khirade MF, Lal G, Bapat SA.	2015	Derivation of a fifteen gene prognostic panel for six cancers	Scientific Reports	5:13248	4.120	19
6.	Kumar B, Uppuladinne MVN, Jani V, Sonavane U, Joshi RR, Bapat SA.	2015	Auto-regulation of SNAI2 mediates its activity during epithelial to mesenchymal transition.	Biochimica et Biophysica Acta (BBA) - Gene Reg. Mech.	1849:1209-1219	5.18	8
7.	Kamble SC, Sen A, Dhake RD, Joshi AN, Midha D, Bapat SA.	2019	Clinical Stratification of High-Grade Ovarian Serous Carcinoma Using a Panel of Six Biomarkers	Journal of Clinical Medicine	8: E330	5.583	2
8.	Varankar SS, Bapat SA.	2018	Migratory Metrics of Wound Healing: A Quantification Approach for *in vitro* Scratch Assays.	Front Oncol.	8: 633	4.137	6
9.	Varankar SS, Bapat SA.	2019	Uncoupling Traditional Functionalities of Metastasis: The Parting of Ways with Real-Time Assays.	J Clin Med.	8: 941	3.303	0
10.	Varankar SS, More M, Abraham A, Pansare K, Kumar B, Narayanan NJ, Jolly MK, Mali AM, Bapat SA.	2020	Functional balance between Tcf21-Slug defines cellular plasticity and migratory modalities in high grade serous ovarian cancer cell lines	Carcinogenesis	41:515-526	5.072	6

Highlighting the important discoveries/contributions described in them briefly –

1. **Bapat SA, Mali AM, Koppikar CB, Kurrey NK. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Research. 2005; 65:3025-9.**

   This was the first global report of the putative identification and isolation of cancer stem cells (CSCs) in ovarian cancer. The germ of the idea that EMT may be associated with CSCs was seeded during the data generated in this study when it was observed that the expression of Snail and Slug in some clones assigned them 'stem cell-like' features. Such an observation was not documented at the time and was completely novel.

   This research report remains my highest cited article > 500 citations.

2. **Kurrey NK, K A, Bapat SA. Snail and Slug are major determinants of ovarian cancer invasiveness at the transcription level. Gynecol Oncol. 2005; 97:155-65.**

   Ectopic expression of Snail or Slug resulted in epithelial–mesenchymal transition (EMT), confirmed through the downregulation of the cytoskeletal component Cytokeratin 18 and upregulation of Vimentin. At a functional level, this correlates with enhanced in vitro clonogenecity, motility and wound healing, and *in vivo* tumorigenecity, invasion and metastases. Snail suppresses expression of adherens and tight junction components, while Slug suppresses expression of all the three junction components; concertedly, bringing down the intercellular adhesion between cells. Further activation of these transcriptional factors in hypoxic conditions revealed a rapid upregulation of Slug expression as an immediate reaction that probably triggers off a signaling cascade leading to Snail expression. This suggested that Slug and Snail may have common as well as distinct roles in ensuring tumor cell survival by signaling the onset of adverse conditions and mediating EMT.

   The study was published in **Gynecologic Oncology** in 2005 and has received over 200 citations.

3. Kurrey NK, Jalgaonkar SP, Joglekar AV, Ghanate AD, Chaskar PD, Doiphode RY, Bapat SA. Snail and Slug mediate radio- and chemo-resistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. Stem Cells. 2009 27(9):2059-2068.

   A genome-wide profiling of stress-triggered EMT-TF target modulation was performed and led to a mechanistic understanding of CSC enrichment following chemotherapy. The EMT-TFs besides driving cell migration also 'de-repress' self-renewal molecules including Nanog and *KLF4*, and supports resistance to p53-mediated apoptosis that together lead to acquisition of a 'stem-like' state by tumor cells. The study complemented a study from Prof. Robert Weinberg's lab that implied another EMT-TF *viz.* Twist in the dedifferentiation f tumor cells to stem cells (2008). Our findings published in **Stem Cells** in 2009 were widely appreciated, and have received 495 citations.

4. Gardi NL, Deshpande TU, Kamble SC, Budhe SR, Bapat SA. Discrete molecular classes of ovarian cancer suggestive of unique mechanisms of transformation and metastases. **Clinical Cancer Research,** 2014,20:87-99.

We further directed these findings to interpret intra-tumor heterogeneity as a determinant of varying cell compositions and molecular expression patterns, which not only could lead to targeted treatments. Applying systems-driven analyses to epigenetic, expression and proteomic signatures resolved discrete molecular HGSC tumor classes, each with unique regulatory networks, pathways and modes of metastases. Notably, a defining feature of one of these classes was Slug-driven EMT, while a second class presented a strong epithelial identity that resisted EMT. The latter was a novel finding and suggested some of the regulatory mechanisms of EMT including the epithelial transcription factor TCF21. These findings were published in **Clinical Cancer Research** in 2014.

5. Khirade MF, Lal G, Bapat SA., Derivation of a fifteen gene prognostic panel for six cancers., **Scientific Reports.** 5, 2015; pp: 13248.

   A pan-cancer a nalysis of gene expression datasets in eleven cancer types identified several components of EMT within modules of highly correlated genes and interactive networks conserved across glioblastoma, breast, ovary, colon, rectal and lung cancers. The specific conserved gene modules were validated across different microarray platforms and datasets; preserved genes within these modules defined regulatory networks associated with metastases, cell migration, metastases, oncogenic transformation, and resistance to apoptosis and senescence, with immune regulation and with PRRX1 being suggested to be master regulator governing the EMT module that includes several TFs and extracellular matrix molecules. Correlation analysis further identified a panel of 15 risk genes with potential prognostic value, termed as the GBOCRL-IIPr panel [(GBM-Breast-Ovary-Colon-Rectal-Lung)–Immune–Invasion–Prognosis], that can potentially be integrated in predicting patient outcomes in the six cancers.

6. Kumar B, Uppuladinne MVN, Jani V, Sonavane U, Joshi RR, Bapat SA. Auto-regulation of SNAI2 mediates its activity during epithelial to mesenchymal transition. **Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms,** 1849, 2015; pp. 1209-1219.

   Slug is a five C2H2 zinc finger (ZF) motif transcription factor. At the molecular level, its functioning involves recognition and interactions with a E-box (CACC/GGTG) consensus elements within target gene promoters to achieve transcriptional repression. However, precise elucidation of events involved in this DNA recognition and binding of specific promoters to regulate target genes have not been achieved. In this study, we demonstrated that besides transcriptional repression in mediating EMT and stemness under conditions of stress, Slug can also directly activate its own expression by preferential binding to specific E-box elements in the distal binding region of its promoter. Our findings suggest that while the first ZF does not contribute to the transcription-associated functions of Slug, all the remaining four ZFs are involved in regulating the expression of target geneswith ZF3 and ZF4 being more crucial than ZF2 or ZF5. We also report that recognition and binding preferences of ZFs are defined through intrinsic differences in the E-box core base pairs and/or flanking sequences, with the S2 E-box element being most critical during autoregulation. However, specific target E-box recognition and binding are also defined by the cellular context, which implies that in silico and/or biochemical DNA binding preferences may not necessarily be able to accurately predict in situ events. Thereby, this study constitutes a novel understanding of transcriptional regulation, and was published in **Biochimica et Biophysica Acta (BBA) - Gene Reg. Mech** in 2015.

7. Kamble SC, Sen A, Dhake RD, Joshi AN, Midha D, Bapat SA. Clinical Stratification of High-Grade Ovarian Serous Carcinoma Using a Panel of Six Biomarkers. **J Clin Med**. 2019 Mar 8;8(3). pii: E330. doi: 10.3390/jcm8030330.

   The clinical relevance of the molecular stratification was explored through collaboration with well-established pathologists from AFMC, KEM and Inlaks-Budhrani Hospital (Pune) and TMC (Kolkata). Development of standard operating protocols (SOPs) for immunohistochemistry - based detection of a panel of 6 biomarkers identified from the systems networks along with a robust scoring system for quantifying their expression in FFPE sections of patient derived tumors was undertaken. This led to a successful validation of the predicted HGSC sub-types at the clinical level, and further revealed transcriptional heterogeneity mediating cellular plasticity and class-switching following chemotherapy. These findings are exciting since they not only validated predicted HGSC stratification at a clinical level, but support the future development of class-specific therapy. The collaborative study was published in **Journal of Clinical Medicine** in 2019.

8. Varankar SS, Bapat SA. Migratory Metrics of Wound Healing: A Quantification Approach for *in vitro* Scratch Assays. Front Oncol. 2018 Dec 18;8:633. doi: 10.3389/fonc.2018.00633. eCollection 2018.

   Experimental approaches to detect metastatic dissemination of tumor cells employ several *in vitro* and *in vivo* assays toward quantification of these functionalities. Most of these endpoint assays rely on the efficacy of wound closure and thwart quantification of migratory phenotypes observed during metastatic dissemination. In this study, we corroborated live cell imaging with the *in vitro* scratch assay toward quantification of migratory modalities in transformed cells. This was achieved through development of a protocol of live cell imaging of the classical wound healing assay, and detailed analyses toward definition of three quantitative metrics viz., displacement, velocity and number of nearest neighbors, which provided global/single-cell resolution of migratory phenotypes as opposed to the classical endpoint assays. EMT *vs.* cooperative cell migration (CCM) These findings were strongly substantiated during an exploration of CCM and EMT as derivatives of three quantitative metrics viz., cell displacement - velocity and number of nearest neighbors during live imaging of cell migration and invasion; these findings provide a highly precise dissection of molecular networks associated with tumor behavior. Routine application of this protocol in cancer biology can aid the design of therapeutic regimes targeting specific migratory modalities and significantly contribute to the dissection of associated molecular networks.

9. Varankar SS, Bapat SA. Uncoupling Traditional Functionalities of Metastasis: The Parting of Ways with Real-Time Assays. **J Clin Med.** 2019 Jun 28;8(7). pii: E941.

   We wrote this review because we felt we need to emphasize and change the current dogma of performing end-point based invasion and migration assays. The inclusion of time lapse microscopy and microfluidic devices in routine assays has recently discerned several nuances of the metastatic cascade. Our review emphasizes that a complete comprehension of metastasis in view of evolving ideologies necessitates (i) the use of appropriate, context-specific assays and understanding their inherent limitations; (ii) cautious derivation of inferences to avoid erroneous/overestimated clinical extrapolations; (iii) corroboration between multiple assay outputs to gauge metastatic potential; and (iv) the development of protocols with improved in situ implications. We further believe that the adoption of improved quantitative approaches in these assays can generate predictive algorithms that may expedite therapeutic strategies targeting metastasis via the development of disease relevant model systems. Such approaches could potentiate the

restructuring of the cancer metastasis paradigm through an emphasis on the development of next-generation real-time assays.

10. Varankar SS, More MM, Abraham A, Kumar B, Narayanan NJ, Jolly MK, Bapat SA. Functional Balance between TCF21-Slug defines phenotypic plasticity and migratory modalities in high-grade serous ovarian cancer cell lines; **Carcinogenesis**. 2020 Jun 17;41(4):515-526.

    Our study identifies novel regulatory cross-talks between Tcf21 and Slug in mediating phenotypic and migration plasticity in HGSC. Differential expression and subcellular localization associate Tcf21, Slug with epithelial, mesenchymal phenotypes, respectively; however, gene manipulation approaches identify their association with additional intermediate phenotypic states, implying the existence of a multistep epithelial-mesenchymal transition program. Tcf21–Slug balance identified across a phenotypic spectrum in HGSC cell lines, associated with microenvironment-induced transitions and the emergence of an epithelial phenotype following drug exposure. Phenotypic transitions and associated functionalities following drug exposure were affirmed to ensue from occupancy of Slug promoter E-box sequences by Tcf21. Our study effectively provides a framework for understanding the relevance of ovarian cancer plasticity as a function of two transcription factors.