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A Mathematical Model for Diagnostic and Prognostic Implications of Micro-RNAs In Human Hepatocellular Carcinoma By Using Weibull Two-Parameter Survival Model.

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Abstract

MicroRNAs (miRNAs) are important gene regulators, which are often deregulated in cancers. In this study, we analyzed the microRNAs profiles of 78 matched cancer/noncancerous liver tissues from HCC patients and 10 normal liver tissues and found that 69 miRNAs were differentially expressed between hepatocellular carcinoma (HCC) and corresponding noncancerous liver tissues (N).Then the expressions of 8 differentially expressed miRNAs were validated by real time RT PCR.The set of differentially expressed miRNAs could distinctly classify HCC,N and normal liver tissues (NL).Moreover, some of these differentially expressed miRNAs were related to the Medical factors of HCC patients. The paper further examines the feasibility of a subfamily of Weibull model. This feasibility is judged based on Bayes information criterion by comparing the Weibull model with its subfamily. Finally we conclude our Mathematical results that the figures 1- A,figure 1-B, figure 1-C is well fitted in the Weibull survival model and the maximum value of SK-Hep-1, Hep G2 and SMMC 7721 at the time has been obtained .This will be helpful for the medical professional.

Keywords: Hepatocellular Carcinoma, microRNA, Weibull distribution.

Mathematical subject classification: 60 G_{xx}, 62H_{xx}, 62$P_{xx.}$

I. Applications

MicroRNAs (miRNAs) are defined as a class of naturally occurring, 19-25 nt long noncoding single-stranded RNA species, which are cleaved from 70-80 nt microRNA precursor transcripts [1]. MiRNAs can regulate gene expression either at the transcriptional or at the translational levels, based on specific binding to the complementary sequence in coding or noncoding region of miRNA transcripts [2]. Recent findings, based on microarray analysis of global miRNA expression profiles in cancer tissues versus normal counterparts, have revealed that miRNA profiles could discriminate malignancies of breast, lung, pancreas, liver and leukemia from normal counterpart [5, 17, 20, 28]. More notably, some specific miRNAs have been reported with predictive significance for prognosis of lung cancer and lymphocytic leukemia [4, 24, 29], thus indicating that miRNAs may play crucial roles in human oncogenesis. Hepatocellular carcinoma (HCC) is one of the most prevalent and lethal cancers in East Asia and South Africa.Murakami et al. reported that miRNAs could discriminate HCC from noncancerous liver tissues (N) in 25 paired samples [20]. Recently, Jiang et al [12] and Budhu et al [3]. In this study, we described miRNA global microarray analysis of 78 HCC and corresponding noncancerous liver tissues as well as 10 normal liver tissues (NL).The miRNA expression profiles could discriminate HCC from both noncancerous and normal liver tissues, and a single miRNA miR-125b can provide predictive significance for prognosis of HCC patients.

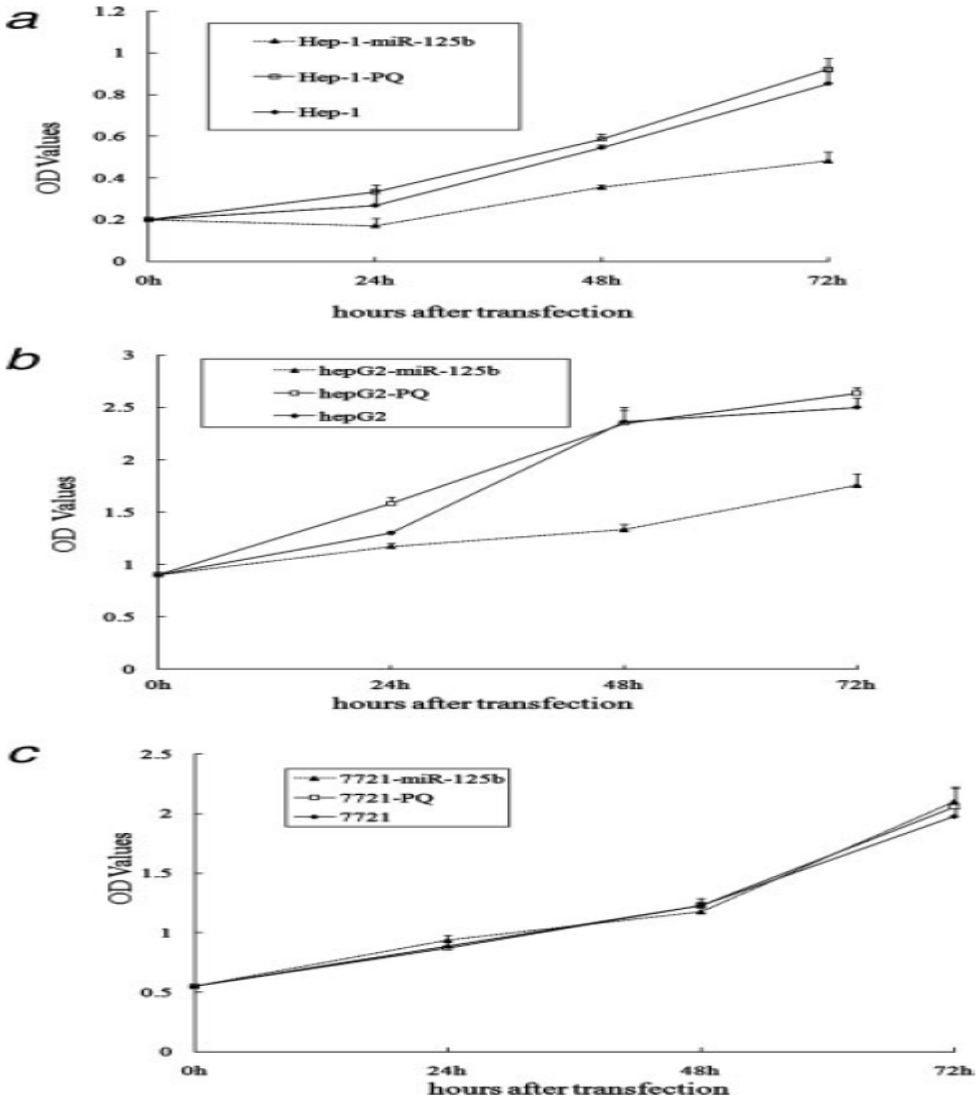


Figure: 1 Hsa-miR-125b could suppress the growth of liver cancer cells.SK-Heb-1 (a),Heb G2 (b) and SMMC 7721 (c) cells were transfected with pQXIN vector (control).Cells were seeded into triplicate wells of 24 well culture plates on day 0.Adherent cells were counted on 24,48 and 72 hr after transfection.Mean values are plotted as shown; error bars represent the standard deviation of mean values from 3 independent experiments.

II. Mathematical Model

The p.d.f of two-parameter Weibull distribution can be written as

$$f(t/\alpha,\beta) = \frac{\beta}{\alpha}\left(\frac{t}{\alpha}\right)^{\beta-1} \exp\left[-\left(\frac{t}{\alpha}\right)^{\beta}\right], \alpha,\beta>0 \quad \text{---------(1)}$$

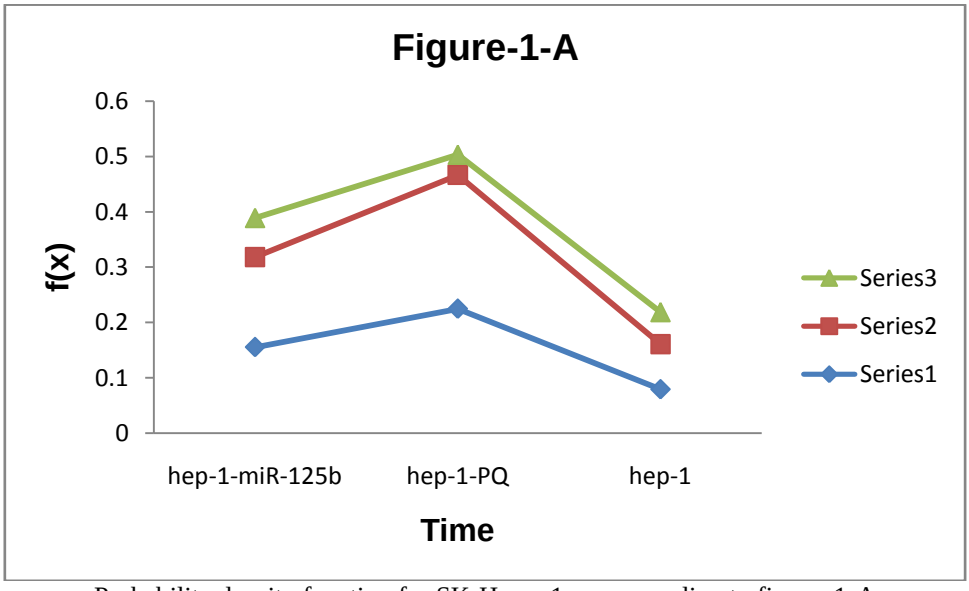
where α is the scale parameter and β determines the shape of the distribution. The corresponding survival function can be given by

$$s(t/\alpha,\beta) = \exp\left[-\left(\frac{t}{\alpha}\right)^{\beta}\right], \alpha,\beta>0\text{----------(2)}$$

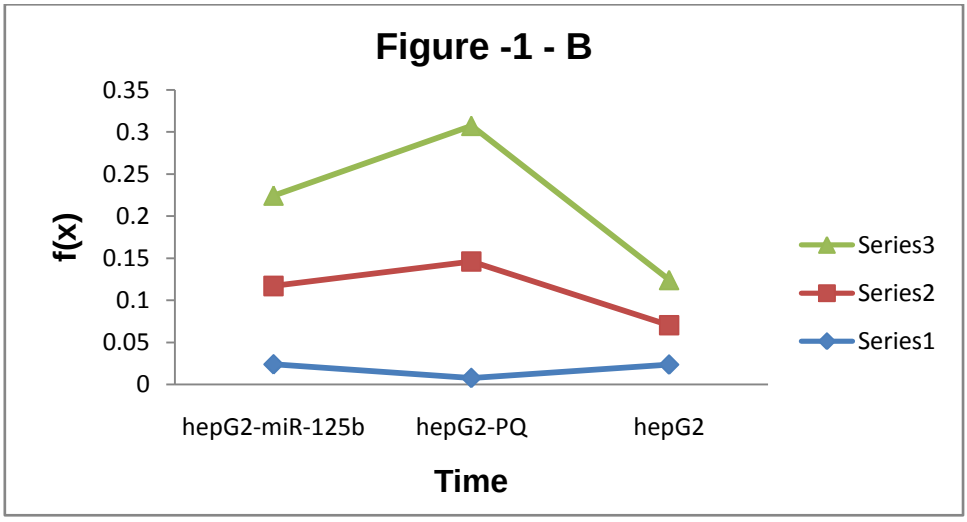
The Weibull distribution encompasses monotonically increasing (for β>1), decreasing (for β<1), and constant (for β=1) failure rate and, as such, the model has been successfully used to describe both initial failures as well as the failures due to remission or aging [16]. One of the biggest advantages with the Weibull model is the availability of closed form survival function, which makes the inferences related to the model quite easy although the non-availability of sufficient statistics poses some problem in comparison to those situations where the existence of the same is guaranteed. The Weibull model is perhaps the richest one as far as the inferential developments are concerned both with regard to classical and Bayesian paradigms.[16] is an important text which systematically describes the classical developments based on the model both in the context of engineering and medical applications. A few other important references include [14]**,** [18] and, more

recently, in [23].The other important references include [8],[10]. The distribution is, in general, not too straightforward to deal with. The classical developments on the model mostly relied on large sample approximations or empirical results. The problem with the Bayesian inferences lies in the involvement of integrals in the posterior based inferences, which are difficult to solve analytically and, as such, require specialized techniques of Bayesian computation [6, 26].This last reference advocated the use of sample based approaches in Bayesian computation because of their several inherent advantages. A few such advantages may include the straightforwardness of the procedures to deal with censored data problems and routine inferential development for some nonlinear functions of the model parameters. The Weibull distribution becomes straightforward if one is confronted with a situation where shape parameter β can be taken to be unity. The resulting distribution becomes one-parameter exponential and inferential developments based on it are routine. This is equivalent to say that an experimenter tests β against unity for the given data set and goes for the exponential model if the hypothesis is accepted. Such problems have been considered earlier by a number of authors in both classical and Bayesian paradigms. The most frequently used classical tool for testing β against unity is based for the Weibull model. For Bayesians, the obvious techniques can be based on the evaluation of Bayes factor which is a bit difficult when the priors are non-informative and the data are compounded with censoring mechanism [25]. The problem of testing $\beta = 1$ can also be visualized as that of model. No doubt, this measure is comparatively easy and provides answers parallel to that based on Bayes factor. A model comparison is justified among the compatible models only where compatibility is referred to mean that all the models under considerations do provide an adequate representation to the given data. Therefore, we first propose a compatibility study of the exponential and Weibull models and then provide a model comparison study to pick up an appropriate on Bayesian version of chi-square discrepancy measure.The posterior corresponding the exponential distribution when $\beta = 1$ has also been commented briefly. Results based on exponential modeling assumption have also been given for completeness.

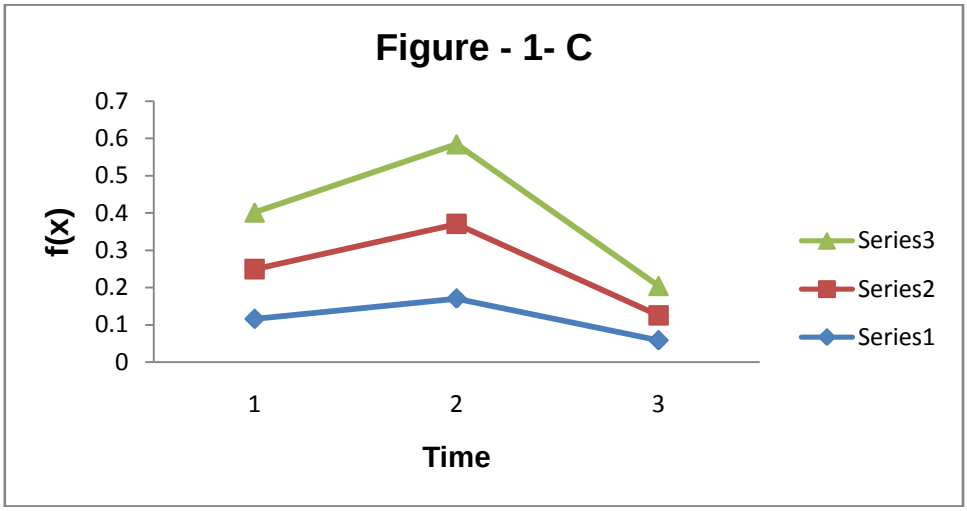
III.Mathematical Results



Probability density function for SK-Hep – 1 corresponding to figure 1-A



Probability density function for Hep G2 corresponding to figure 1-B



Probability density function for SMMC 7721 corresponding to figure 1-C

IV. Discussion

In this study, 78 pairs of HCC and matched noncancerous tissues and 10 normal liver tissues were used for miRNAs microarray assays. From our data, the differential expressed miRNAs could distinctly discriminate HCC from their matched noncancerous liver and 10 normal liver tissues. Additionally we have to mention that the miRNA profile we found to be diagnostic for HCC was partially consistent with those reported by Murakami [20].Among their 7 top nature miRNAs,5 (mir-18,mir-224,mir-199a,mir-199a*,mir-195) were also among miRNAs we found to be differentially expressed in HCC versus noncancerous liver. The discrepancy in other miRNAs may be attributed to the different miRNA probes we used or the different etiological factors involved in HCC comprised Aflatoxin and hepatitis B virus infection, which accounted for more than 84% of HCC cases. Also the difference in genetic background between these ethnic groups remained unclear.Therefore, the partial difference in miRNA species involved in HCC from 2 countries was not expected. Besides, expression of hsa-miR-122a was down regulated in hepatocellular carcinoma in our study, as reported by Gramantieri et al [9].and Kutay et al [15]. Among the HCC discriminative signature miRNAs in our studies, some miRNAs may be commonly deregulated in other cancers derived from different tissues reported previously. For example,hsa-miR-221 and hsa-miR-222 were found deregulated in thyroid papillary carcinomas;has-miR-181a in glioblastoma;hsa-miR-15b,hsa-miR-107 and Let-7 in acute promyelocytic leukemia [7]. Taken together, miRNA species deregulated commonly in diverse type of cancers might possibly play crucial roles in human carcinogenesis and cancer progression. Although miRNA profile did reveal very prospective features

in cancer, the functions and real targets of miRNAs were largely unknown.Recently; Slack's group has proved that the Let-7 family could down regulate RAS in C.elegans as well as in human cells [13]. In addition, the PTEN gene was targeted by hsa-mir-21[24], cyclin G1 by hsa-mir-222. The predicted targets of the majority of microRNAs based on sequence homology remained to be comprehensively validated by in vitro and in vivo experiments. The molecular events underlying the altered miRNA profiles in HCC need to be further extensively investigated by using conventional gene expression microarray and proteomic analysis.The most important finding in our study is the discovery of the specific miRNA related to prognosis of HCC patients, hsa-miR-125b.The expression level of hsa-miR-125b in HCC tissues is generally downregulated as compared with that of noncancerous liver tissues. On the basis of the survival analysis, we found that HCC patients with high expression of hsa-miR-125b had good prognosis, while those with low expression gave poor clinical outcomes. By the reports, miR-125b were commonly deregulated in cancers derived from different histotypes, such as prostate cancer, breast cancer and thyroid anaplastic carcinomas [11, 21, 27]. Furthermore, depletion of miR-125b had a profound effect on the proliferation of adult differentiated cancer cells.Overexpression of miR-125b could suppress and invasion phosphorylation of ERK1/2 and Akt and reduce migration and invasion capacities of human breast cancer cell [22]. Besides, miR-125b played an important role in innate immune response. So these reports suggested the importance of miR-125b in cancers.But,to the best of our knowledge, there is no report so far on the function of hsa-miR-125b in HCC.Our studies showed that miR-125b obviously suppressed growth of hepatoma cells.Next,we analyzed the putative target of miR-125b and found several serine/theronine protein kinase and related genes were involved, such as ras-related protein Rab-13(RAB13),serine/threonine-protein kinase 13(AURKC),tyrosine-proteinkinase receptor Tie-1 precursor(TIE1) and phosphoinositide 3-kinase regulatory subunit 5(PIK3R5).These results suggested that mir-125b may repress the cell growth by inhibiting the protein kinases.Akt was reported to be the most important downstream signaling mediator of phosphoinositide 3-kinase and crucially controlling cell survival and proliferation. So we further investigated the possible role of miR-125b on phosporylation of Akt.The results showed that miR-125b could obviously suppress the phosporylation of Akt.Summarizing these results, this study implicated that miR-125b might suppress cancer cell proliferation through inactivating Akt.

V. Conclusion

miRNA signature was identified as a HCC diagnostic discriminator from both noncancerous and normal liver tissues. Most importantly, this is the first report to identify single miRNA correlated to the HCC prognosis. that is hsa-miR-125b as a HCC survival predictor,the mir-125 inhibits cell growth and phosporylation of Akt in hepatoma cells. Because a small set of miRNA species could reveal crucial Medical information, these tests could be readily adapted to small scale microchips or nanomere-based assay feasible for Medical application.. Finally we conclude our Mathematical results that the figures 1- A, figure 1-B, figure 1-C is well fitted in the Weibull survival model and the maximum value of SK-Hep-1, Hep G2 and SMMC 7721 at the time has been obtained .This will be helpful for the medical professional.

References

[1]. **Bartel DP**.MicroRNAs**:** genomics, biogenesis, mechanism, and function. Cell 2004:116:281-97.

[2]. **Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM.Bantam** encodes a Developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. Cell 2003:113:25-36.

[3]. **Budhu A,Jia HL,Forgues M,Liu CG,Goldstein D,Lam A,Zanetti KA,Ye QH,Qin LX,Tang ZY,Wang XW,**. Identification of metastasis-related micro RNAs in Hepatocellular carcinoma. Hepatology 2008; 47:897-907.

[4]. **Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M.Wojcik SE, Iorio MV,Visone R,Sever NI,Fabbri M,Iuliano R,Palumbo T,et al.**A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia.N Engl J Med 2005; 353:1793-801.

[5]. **Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD, Shimizu M,Cimmino A,Zupo S,Dono M,Dell' Aquila ML, Alder H, et al**.MicroRNA profiling reveals distinct Signatures in B cell chronic lymphocytic leukemias.Proc Natl Acad Sci USA 2004; 101:11755-60**.**

[6]. **Gamerman D and Lopes H.F.**Markov Chain Monte Carlo:Stochastic Simulation for Bayesian Inference, 2nd ed.,Chapman & Hall / CRC.(2006).

[7]. **Garzon R,Pichiorri F,Palumbo T,Visentini M,Aqeilan R,Cimmino A,Wang H,Sun H,Volinia S,Alder H,Calin GA,Liu CG,et al**.MicroRNA gene expression during retinoic acid-induced

differentiation of human acute promyelocytic leukemia.Oncogene 2007; 26:4148-57.

[8]. **Gelman A.Carlin J.B,Stern H.S,Rubin D.B.** Bayesian Data analysis 2nd ed. Chapman & Hall, London.(2003).

[9]. **Gramantieri L.Ferracin M,Fornari F,Veronsee A, Sabbioni S, Liu CG.Calin GA, Giovannini C,Ferrazzi E,Grazi GL, Crice CM,** Bolondi L,et Cal. yclin G1 is a target of miR-122a, A microRNA frequently down-regulated and in human Hepatocellular carcinomas. Cancer Res 2007; 67:6092-9.

[10]. **Ibrahim J.G.Ming-Hui Chen and Sinha** D.Bayesian methods for joint modeling of longitudinal and survival data with applications to cancer vaccine trials, Statistica Sinica, 14, p.863-883.(2004).

[11]. **Iorio MV, Ferracin M, Liu cg, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S,Palazzo JP,et al**.MicroRNA gene expression deregulation in human breast cancer.Cancer Res 2005; 65:7065-70.

[12]. **Jiang J,Gusev Y.Aderca I,Mettler TA,Nagorney DM,Brakett DJ,Roberts LR, Schmittgen TD**.Association of microRNA expression in hepatocellular Carcinoma with hepatitis infection. Cirrhosis and patient survival .Clin cancer Res 2008; 14:419-27.

[13]. **Johnson SM,Grosshans H,Shingara J,Byrom M, Jarvis R, Cheng A, Labourier e, Reinert KL, Brown D, Slack FJ**,RAS is regulated by the let-7 microRNA family.Cell 2005; 120:635-47.

[14]. **Kalbfleisch J.D and Prentice R.L**. The statistical analysis of failure Time Data. Wiley, New York (2002).

[15]. **Kutay H, Bai S, Datta J, Motiwala T, Pogribny I, Frankel W, Jacob ST, Ghoshal K.** Downregulation of miR-122 In The rodent And Human hepatocellular carcinomas. J Cell Biochem 2006; 99:671-8.

[16]. **Lawless J.F** .Statistical Models and Methods for Lifetime Data.2nd ed., Wiley, New York (2002).

[17]. **Lee EJ,Gusev Y,Jiang J,Nuovo GJ,Lerner MR,Frankel WL,Morgan DL,Postier RG, Brackett DJ, Schmittgen TD.**Expression profiling identifies microRNA Signature in pancreatic cancer.Int J Cancer 2007;120:1046-54.

[18]. **Lee E.T. and Wang J.W**.Statistical methods for survival Data Analysis, Wiley, New York (2003).

[19]. **Murakami Y.Yasud T,Saigo K,Urashima T,Toyoda H,Okanoue T,Shimotohno K.**Comprehensive analysis of microRNA expression patterns in hepatocellular Carcinoma and non-tumorous tissues. Oncogene 2006; 25:2537-45.

[20]. **Ozen M,Creighton CJ,Ozdemir M, Ittmann M**,Widespread deregulation of MicroRNA expression in human prostate cancer.Oncogene 2008; 27:1788-93.

[21]. **Scott GK,Goga A,Bhaumik D,Berger CE,Sullivan CS,Benz CC**.Coordinate Suppression of ERBB2 and ERBB3 by enforced expression of micro-RNA miR 125a or miR-125b.J Biol Chem 2007; 282:1479-86.

[22]. **Singpurwalla N.D**.Reliability and Risk:A Bayesian perspective. Wiley, New York (2006).

[23]. **Takamizawa J,Konishi H,Yanagisawa K.Tomida S,Osada H,Endoh H,Harano T,Yatabe Y,Nagino M,Nimura Y,Mitsudomi T,Takahashi T.**Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened Postoperative survival. Cancer Res 2004; 64:3753

[24]. **Upadhyay S.K and Mukherjee** B.Assessing the value of the threshold Parameters in the Weibull distribution using Bayes paradigm, IEEE Trans.Reliab. (2008) 57.p.489-497.

[25]. **Upadhyay S.K.,Vasistha N and Smith A.F.M**.Bayes inference in life testing and reliability via Markov chain Monte Carlo simulation,Sankhya A, (2001)63,p.15- 40.

[26]. **Visone R,Pallante P, Vecchione A, Cirombella R, Ferracin M, Ferraro A, Volinia S, Coluzzi S, Leone V, Borbone E, Liu CG, Petracca F, et al.**Specific microRNAs are Down reregulated in human thyroid anaplastic carcinomas. Oncogene 2007; 26:7590-5.

[27]. **Volinia s, Calin GA, Liu CG,Ambs s,Cimmino A, Petrocca F, Visone R, lorio M, Roldo C , Ferracin M, Prueitt RL Yanaihara N,et al**. MicroRNA expression Signature of human solid tumors defines cancer gene targets.Proc Natl Acad Sci USA 2006:103:2257-61.

[28]. **Yanaihara N, Caplen N, Bowman E, Seike M,Kumamoto K.Yi M,Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG,et al.**Unique microRNA molecular profiles in lung cancer diagnosis and prognosis.Cancer Cell 2006;9:189-98.