The co-existence of states in p53 dynamics driven by miRNA

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The regulating mechanism of miRNA on p53 dynamics in p53−MDM2−miRNA model network incorporating reactive oxygen species (ROS) is studied. The study shows that miRNA drives p53 dynamics at various states, namely, stabilized states and oscillating states (damped and sustain oscillation). We found the co-existence of these states within certain range of the concentration level of miRNA in the system. This co-existence in p53 dynamics is the signature of the system’s survival at various states, normal, activated and apoptosis driven by a constant concentration of miRNA.

Introduction. – The p53, tumor suppressor protein, attracted the interest of researchers because of its important role in preventing cell to become cancer [1, 2]. It acts as a key regulator in the cellular network and response to a variety of cellular stress, including DNA damage, hypoxia, nucleotide depletion, nitric oxide and aberrant proliferative signals (such as oncogene activation) [1, 8]. But in most cases of human cancer cell, p53 tumor suppressor signaling pathway usually found in inactivated condition [1]. Its activation results in the fulfillment of key cellular processes, for example, cell-cycle arrest, senescence and most importantly tumor clearance to prevent cancer cell formation [4]. Further, activated p53 protein safeguards the organism against the propagation of cells that carry damaged DNA with potentially oncogenic mutations [2]. It has been reported that activation of p53 functions via the inhibition of MDM2 protein can be regarded as an effective approach in cancer therapy [2]. Because MDM2 acts as a negative feedback regulator (inhibitor) to p53 by binding itself to p53, and then physically blocking its ability to transactivate gene expression, and stimulating its degradation [6, 8]. Further, the interaction of N-terminal domain of MDM2 with transactivation domain of p53 (p53-TAD) performs a significant role in the regulation of the G1 checkpoint of the cell cycle and cell function [8, 10].

ROS (Reactive oxygen species) are chemically reactive molecules containing oxygen ions and peroxides [11]. They are synthesized from normal metabolism of oxygen as a natural byproduct and play important roles in cell signaling and homeostasis [12, 13]. However, ROS level inside cell can be elevated by UV irradiation or heat exposure which can drive the cell at different stress states [12]. High level of ROS can promote DNA damage, and may probably lead the cell to mutagenesis, carcinogenesis and aging [11, 13, 14]. However, the role of ROS in driving the cell at different states, namely, normal, stress, cancerous and apoptosis is still not fully studied.

MicroRNAs (miRNAs) are small noncoding RNA molecules of size 20-24 nucleotides, and are powerful regulators of transcriptional and post transcriptional gene expression which regulate both physiological and pathological processes such as cellular development and cancer [15, 17]. miR-125b is a brain-enriched miRNA which acts as a negative regulator of p53 both in zebrafish and human [18, 20]. Overexpression of miR-125b suppresses the endogenous level of p53 protein and represses to apoptosis in human neuroblastoma cells and human lung fibroblast cells [20]. Decrease in level of miR-125b leads to enhance the level of p53 and induces apoptosis in human neuroblastoma and human lung fibroblast cells [18, 20]. However, the regulating mechanism of miR-125b with p53 is not fully studied. The dynamics of p53 and its response to the miR-125b regulation are still open questions. In the present study, we try to answer some of these fundamental questions based on basic model built from available experimental reports.

p53−MDM2−miRNA model.– The model we consider (Fig. 1) is integration of p53-Mdm2 regulatory network [21] with stress inducers ROS via DNA damage [13] and miRNA which interact with p53_MDM2 [19]. In this model we assume that miRNAAs are supposed to be constantly produced in the nucleus either from their own genes or encode from introns (non-coding sequence) with a rate $k_1$ [19]. The synthesis of miRNA model.

The model we consider (Fig. 1) is integration of p53-Mdm2 regulatory network [21] with stress inducers ROS via DNA damage [13] and miRNA which interact with p53_MDM2 [19]. In this model we assume that miRNAAs are supposed to be constantly produced in the nucleus either from their own genes or encode from introns (non-coding sequence) with a rate $k_1$ [19]. ROS synthesis is assumed to occur with a rate of $k_1$. This ROS synthesis triggers DNA damage with a rate of $k_1$. Then this DNA damage leads to the activation of ARF with a rate $k_1$ followed by the degradation of ARF with a rate of $k_1$. Further, the activated ARF protein binds to MDM2 with a rate of $k_2$ to control ubiquitination of p53 [20]. The ARF and MDM2 interaction results into the formation of ARF_MDM2 complex [24]. The formation of ARF_MDM2 complex reduces the concentration level of MDM2 in the systems which in turn alters the behaviour of p53 [24]. On the other hand, dissociation of ARF_MDM2 complex with a rate $k_2$ helps the degradation of MDM2 population and recruit activated ARF. miRNA directly interacts with p53_mRNA to form miRNA_p53_mRNA complex at a rate $k_2$ [17]. The ubiquitination of p53_mRNA is done via miRNA which occurs with a rate $k_3$ [19]. The synthesis of p53 takes place through transcription of p53_mRNA with a rate $k_4$. Further, this p53 synthesis depends on the avail-
The model biochemical network (Fig. 1) described by the twenty two reaction channels (Table 2) can be described by the following coupled ordinary differential equations (ODE) using Mass action law of chemical kinetics,

\[
\frac{dx_i(t)}{dt} = F_i \left[ x_1(t), x_2(t), \ldots, x_N(t) \right]
\]

where, \(i = 1, 2, \ldots, N\) and \(F_i\) is the ith function whose form is given in Supplementary file. The non-linear coupled \(N\) ODEs (1) (Supplementary file) of \(p53-\)MDM2-miRNA model are solved using 4th order Runge-Kutta method which is the standard algorithm for numerical integration [21] to find the dynamics of the system variables. The simulation is done for 10 days using the parameter values given in Supplementary file (Table 2) and starting from an initial condition.

**ROS driven p53 phase transition.**—The concentration of ROS in the system drives the system dynamics at different states which may correspond to various temporal cellular states. The simulation is done first keeping \(k_{\text{miRNA}} = 0\) throughout the numerical experiment, and changing the parameter \(k_{\text{ROS}}\) (Fig. 2). Since \(k_{\text{ROS}}\) is the rate of creation of ROS, the concentration of ROS synthesized in the system is proportional to \(k_{\text{ROS}}\). The \(p53\) level in the system is maintained at stabilized state with minimum concentration level for sufficiently small values of \(k_{\text{ROS}} (k_{\text{ROS}} \leq 0.00002)\) which may correspond to normal state of the system. As the value of \(k_{\text{ROS}}\) increases slightly \((0.0002 \leq k_{\text{ROS}}(0.002))\) the dynamics cross over from stable state to damped oscillation state (Fig. 2 B) where the dynamics preserves stable condition for certain interval of time (\([0-7]\) days), and then it becomes activated (for time \(\geq 7\) days) induced by \(k_{\text{ROS}}\). This result suggests that as the concentration of ROS increases in the system, it causes more DNA damage due to which \(p53\) dynamics become stressed and exhibits an oscillatory pattern. Further increase in the value of \(k_{\text{ROS}} (0.002 \leq k_{\text{ROS}}(0.008))\) leads the \(p53\) dynamics to damped oscillation for some interval of time then to sustained oscillation with increasing amplitude (Fig. 2 C and D; Fig. 3 upper left panel). The sustain oscillation indicates that the \(p53\) is strongly activated (the stress is maximum).

Now, excess increase in ROS concentration \((k_{\text{ROS}} \geq 0.015)\) drives the \(p53\) dynamics from sustain to damped oscillation (Fig. 2 E), after which \(p53\) state is switched to stabilized state (Fig. 2 E and F; Fig. 3 upper left panel). This suggests that extreme values of \(k_{\text{ROS}}\) may cause very high DNA damage, such that the damage could not able be repaired back, which could be the condition of apoptotic phase.

**Role of miRNA on p53 dynamics.**—The interaction of miRNA with \(p53\) is done via \(p53\)-miRNA complex in indirect fashion. The impact of miRNA on \(p53\) was studied by keeping fixed \(k_{\text{ROS}} = 0.00005\) and allowing to change the values of \(k_{\text{miRNA}}\) (Fig. 2 right panels). Similarly, as obtained in ROS case, we get three different states namely stable, damped with sustain oscillating pattern. Further increase in the value of \(k_{\text{ROS}} (0.002 \leq k_{\text{ROS}}(0.008))\) leads the \(p53\) dynamics from sustain to damped oscillation for some interval of time then to sustained oscillation with increasing amplitude (Fig. 2 C and D; Fig. 3 upper left panel). The sustain oscillation indicates that the \(p53\) is strongly activated (the stress is maximum).
and again stable state of p53 driven by miRNA (Fig. 2 right panels). The small values of $k_{miRNA}$ ($k_{miRNA} (0.00001)$ could not able to provide significant stress to p53 dynamics, and maintains at stabilized state (Fig. 2 A). The further increase in $k_{miRNA}$ values ($0.0001 \leq k_{miRNA} (0.0002)$ the dynamics still maintains stability upto certain interval of time (Fig. 2 B, C, D), after which the dynamics is switched to damped oscillation (weakly activated) for short interval of time and then to sustain oscillation (strongly activated). Further increase in $k_{miRNA}$ compels the dynamics to stabilized state again with low concentration level (Fig. 2 F). This suggests that the increase in concentration of $miRNA$ in the system drives the system at various stress states, lowering p53 concentration level $^{20,28}$. The excess $k_{miRNA}$ values induce lowering of p53 concentration level even below normal stabilized p53 state indicating the possibility of switching stress state to cancerous state $^{28}$.

**Co-existence of states.**— The phase transition like behaviour of the system dynamics induced by ROS and miRNA concentrations available in the system can be well characterized by analysing the nature of transition time of the p53 dynamics. We define $T_{1s}$ to be the transition time below ($t(T_{1s})$) which the dynamics shows stable state (does not show any oscillation) and above which the dynamics shows oscillatory behaviour. We further define second transition time, $T_{ds}$ which separates increasing damped and sustain oscillations (Fig. 2). Similarly, $T_{sd}$ and $T_{2s}$ are taken as transition times separating sustain and damped oscillation, and damped oscillation and stabilized state. We then calculated $T_{1s}$, $T_{ds}$, $T_{sd}$ and $T_{2s}$ as a function of $k_{ROS}$ (Fig. 4 upper panel) where the regimes for $T(T_{1s}$ and $T_{2s}$) corresponds to stabilized states, regimes between $T_{ds}$, $T_{sd}$ and $T_{2s}$ corresponds to damped states and $T_{sd}$ and $T_{ds}$ indicates the sustain oscillation state regime.

The results indicate that there is a certain range of $k_{ROS}$ (region bounded by two lines) where one can find the four states together including two stable states for

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**FIG. 2.** (A) The p53 temporal behaviour when ROS act as a stress inducer whereas $miRNA$ concentration rate kept fixed. (B) The p53 temporal behaviour when $miRNA$ act as a stress inducer whereas ROS creation rate kept fixed.

**FIG. 3.** A comparative plot for the amplitude verses $k_{ROS}$ in first panel and $k_{miRNA}$ in 2nd panel. Similarly, comparative plot for the time period variation verses $k_{ROS}$ in 3rd panel and $k_{miRNA}$ in 4th panel.

**FIG. 4.** A phase diagram showing impact of $k_{ROS}$ on stability of p53 as well as impact of $k_{miRNA}$ on stability of p53 protein.
that concentration of cancerous state. Various experimental studies reported miRNA provide many hidden information regarding the activity of [18, 20]. The obtained results are quite interesting and to study uterus cancer cell lines. Therefore, it is very important roles of it in regulating cancerous cells.

Conclusions.— p53 is found to be a versatile protein which can interact with a number of protein and participate in many biologically important pathway. There are a number of factors which can induce cellular stress, such as environmental factors (UV, IR etc), stress inducing molecules (ROS, miRNA, nitric oxide and many other molecules). The variation in concentration of reactive oxygen species in cellular system leads to the changes in the p53 dynamics (various stress states) with overall enhancement in its concentration level in the cell. Further, the introduction miRNA 125b to the system shows inhibitory effect on p53 production and switching of stress states by varying miRNA 125b concentration [18]. The obtained results are quite interesting and provide many hidden information regarding the activity of miRNA 125b that it can probably switch the system to cancerous state. Various experimental studies reported that concentration of miRNA 125b increases in different cancer cell lines especially in breast cancer, leukemia and uterus cancer cell lines. Therefore, it is very important to study miRNA in depth in order to understand other roles of it in regulating cancerous cells.

Our study shows that significant activity of miRNA can be seen only when the the system is slightly activated by ROS but this process is not needed to study ROS activity. This means that there is always a competition between ROS and miRNA which is needed to be investigated extensively. Moreover, the impact of the miRNA on p53 regulatory pathway should be further studied in stochastic system in order to capture the state switching mechanism quantitatively and to understand the role of noise in the cellular process.

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The co-existence of states in $p53$ dynamics driven by $miRNA$

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The model biochemical network (Fig. 1) described by the twenty two reaction channels (Table 2) can be described by the following coupled ordinary differential equations (ODE) using Mass action law of chemical kinetics,

$$\frac{dx_1}{dt} = k_4x_4 - k_{10}x_1x_2 + k_{12}x_5$$ (1)

$$\frac{dx_2}{dt} = k_8x_3 - k_{10}x_1x_2 + k_{11}x_5 + k_{12}x_5 - k_{13}x_2 - k_{20}x_4$$ (2)

$$\frac{dx_3}{dt} = k_7x_1 - k_9x_3$$ (3)

$$\frac{dx_4}{dt} = -k_2x_1x_4 + k_5 - k_6x_4$$ (4)

$$\frac{dx_5}{dt} = k_{10}x_2x_1 - k_{11}x_5 - k_{12}x_5$$ (5)

$$\frac{dx_6}{dt} = k_{14} - k_{15}x_6 - k_{16}x_6$$ (6)

$$\frac{dx_7}{dt} = k_{16}x_6 - k_{17}x_7$$ (7)

$$\frac{dx_8}{dt} = k_{18}x_7 - k_{19}x_8 - k_{20}x_8x_2 + k_{21}x_9$$ (8)

$$\frac{dx_9}{dt} = k_{20}x_8x_2 - k_{21}x_9$$ (9)

$$\frac{dx_{10}}{dt} = k_1 - k_{22}x_1x_4 + k_3x_{11} - k_{22}x_{10}$$ (10)

$$\frac{dx_{11}}{dt} = k_2x_1x_4 - k_3x_{11}$$ (11)

The set of ODEs can be written in compact form as in the following,

$$\frac{dx(t)}{dt} = F(x_1, x_2, \ldots, x_N)$$ (12)

where, $F = [F_1, F_2, \ldots, F_N]^T$ is the functional vector.

The time evolution of the state vector $\vec{x}(t)$ can be obtained by numerically solving the non-linear coupled differential equations (1)-(11) using standard 4th order Runge-Kutta algorithm for numerical integration [27].

**Stability analysis**

The fixed or equilibrium points of the ODE given by equation (12) can be obtained by putting $\frac{dx(t)}{dt} = 0$ and solving for $x_1^\ast$, $x_2^\ast$, $\ldots$, $x_N^\ast$ from these equations. In our model described by mathematical equations (1)-(11), we have the following equilibrium points,

$$x_1^\ast = \left[ \begin{array}{c} k_1k_5k_9 \k_7k_8k_{10}k_{11} \{k_{13} + k_{\text{ROS}}k_{16}k_{18}k_{20} \} \k_6 + \frac{k_5}{k_{22}}k_{\text{miRNA}} \end{array} \right]^{1/2}$$

$$x_2^\ast = \left[ \begin{array}{c} k_4k_5k_7k_8(k_{11} + k_{12}) \k_9k_{10}k_{11} \{k_{13} + k_{\text{ROS}}k_{16}k_{18}k_{20} \} \k_6 + \frac{k_5}{k_{22}}k_{\text{miRNA}} \end{array} \right]^{1/2}$$

$$x_3^\ast = \left[ \begin{array}{c} k_4k_5k_7k_8 \{k_{13} + k_{\text{ROS}}k_{16}k_{18}k_{20} \} \k_6 + \frac{k_5}{k_{22}}k_{\text{miRNA}} \end{array} \right]^{1/2}$$

$$x_4^\ast = \left[ \begin{array}{c} k_4k_5k_7 \k_k_{16}k_{18} \{k_{13} + k_{\text{ROS}}k_{16}k_{18}k_{20} \} \k_6 + \frac{k_5}{k_{22}}k_{\text{miRNA}} \end{array} \right]^{1/2}$$

$$x_5^\ast = \left[ \begin{array}{c} k_4k_5k_7 \k_k_{16}k_{18} \{k_{13} + k_{\text{ROS}}k_{16}k_{18}k_{20} \} \k_6 + \frac{k_5}{k_{22}}k_{\text{miRNA}} \end{array} \right]^{1/2}$$

$$x_6^\ast = \left[ \begin{array}{c} k_4k_5k_7 \k_k_{16}k_{18} \{k_{13} + k_{\text{ROS}}k_{16}k_{18}k_{20} \} \k_6 + \frac{k_5}{k_{22}}k_{\text{miRNA}} \end{array} \right]^{1/2}$$

$$x_7^\ast = \left[ \begin{array}{c} k_4k_5k_7 \k_k_{16}k_{18} \{k_{13} + k_{\text{ROS}}k_{16}k_{18}k_{20} \} \k_6 + \frac{k_5}{k_{22}}k_{\text{miRNA}} \end{array} \right]^{1/2}$$

$$x_8^\ast = \left[ \begin{array}{c} k_4k_5k_7 \k_k_{16}k_{18} \{k_{13} + k_{\text{ROS}}k_{16}k_{18}k_{20} \} \k_6 + \frac{k_5}{k_{22}}k_{\text{miRNA}} \end{array} \right]^{1/2}$$

$$x_9^\ast = \left[ \begin{array}{c} k_4k_5k_7 \k_k_{16}k_{18} \{k_{13} + k_{\text{ROS}}k_{16}k_{18}k_{20} \} \k_6 + \frac{k_5}{k_{22}}k_{\text{miRNA}} \end{array} \right]^{1/2}$$

$$x_{10}^\ast = \left[ \begin{array}{c} k_1k_2 \k_k_{16}k_{18} \{k_{13} + k_{\text{ROS}}k_{16}k_{18}k_{20} \} \k_6 + \frac{k_5}{k_{22}}k_{\text{miRNA}} \end{array} \right]^{1/2}$$

The stabilized state of $p53$ ($x_1^\ast$) and Mdm2 ($x_2^\ast$) are dependent on the values of the parameters $k_{\text{miRNA}}$ and $k_{\text{ROS}}$, and there is competition between these two parameters affecting stabilized states of $p53$ and Mdm2. Keeping $k_{\text{miRNA}}$ to a constant value, equation (13) shows that $x_1^\ast \propto \sqrt{1 + A k_{\text{ROS}}}$, where $A = \frac{k_3k_6}{k_{13}k_{17}k_{18}k_{15}k_{16}}$, which drives the low equilibrium state (may be normal state where $x_1^\ast$ is maintained minimum value) at low $k_{\text{ROS}}$ to the higher equilibrium state (may be apoptosis state where $x_1^\ast$ is maintained high value) at high $k_{\text{ROS}}$ increases. However, in the case of Mdm2, the scenario is opposite, where $x_2^\ast \propto \frac{1}{\sqrt{1 + A k_{\text{ROS}}}}$, and $k_{\text{ROS}}$ drives the higher Mdm2 equilibrium state to lower equilibrium state.

Further, if $k_{\text{ROS}}$ and other rates are kept constant, $x_1^\ast \propto \frac{1}{\sqrt{B + k_{\text{miRNA}}}}$, and $x_2^\ast \propto \frac{1}{\sqrt{B + k_{\text{miRNA}}}}$, where $B$ is a
constant given by $B = \frac{k}{k_{miRNA}}$. This means that as $k_{miRNA}$ increases, $k_{miRNA}$ drives the higher equilibrium state of both p53 and Mdm2 to lower equilibrium states.

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| S.No | Species Name                  | Description                               | Notation |
|------|------------------------------|-------------------------------------------|----------|
| 1.   | p53                          | Unbounded p53 protein                     | x₁       |
| 2.   | Mdm2                         | Unbounded Mdm2 protein                    | x₂       |
| 3.   | Mdm2_mRNA                    | Mdm2 messenger mRNA                      | x₃       |
| 4.   | p53_mRNA                     | p53 messenger mRNA                       | x₄       |
| 5.   | Mdm2 − p53                   | Mdm2 with p53 complex                    | x₅       |
| 6.   | ROS                          | Reactive Oxygen Species                  | x₆       |
| 7.   | Dam_DNA                      | Damage DNA                               | x₇       |
| 8.   | ARF                          | Alternative Reading Frame protein        | x₈       |
| 9.   | ARF_Mdm2                     | ARF and Mdm2 complex                     | x₉       |
| 10.  | mi − RNA − 125b              | Micro RNA 125b                           | x₁₀      |
| 11.  | mi − RNA − p53 − mRNA        | Micro RNA 125b and p53_mRNA complex      | x₁₁      |

Table 1 - List of molecular species

| S.No | Reaction                              | Name of the process                             | Kinetic Law | Rate Constant | References |
|------|---------------------------------------|-------------------------------------------------|-------------|---------------|------------|
| 1.   | φ k₁ → x₁₀                            | Micro RNA creation                              | k₂          | 1 × 10⁻⁴ sec⁻¹ | 16, 14, 20 |
| 2.   | x₁₀ + x₄ k₂ → x₁₁                    | Synthesis of miRNA and p53_mRNA complex        | k₂(x₁₀)(x₄) | 2 × 10⁻² sec⁻¹ | 16, 24     |
| 3.   | x₁₁ k₃ → x₁₀                          | miRNA_p53_mRNA degradation                     | k₃(x₁₁)     | 1 × 10⁻⁴ sec⁻¹ | 16, 24     |
| 4.   | x₄ k₄ → x₁ + x₄                       | p53 mRNA translation                           | k₄(x₄)      | 8 × 10⁻² sec⁻¹ | 3, 21     |
| 5.   | φ k₅ → x₄                            | p53_mRNA synthesis                             | k₅          | 1 × 10⁻³ sec⁻¹ | 3, 21     |
| 6.   | x₄ k₆ → φ                            | p53_mRNA degradation                           | k₆(x₄)      | 1 × 10⁻⁴ sec⁻¹ | 3, 21     |
| 7.   | x₁ k₇ → x₁ + x₃                       | Mdm2_mRNA synthesis                            | k₇(x₁)      | 1 × 10⁻⁴ sec⁻¹ | 3, 21, 27 |
| 8.   | x₃ k₈ → x₂ + x₃                       | Mdm2 synthesis                                 | k₈(x₃)      | 495 × 10⁻⁵ sec⁻¹ | 3, 21, 27 |
| 9.   | x₃ k₉ → φ                            | Mdm2_mRNA degradation                          | k₉(x₃)      | 1 × 10⁻⁴ sec⁻¹ | 3, 21, 27 |
| 10.  | x₁ + x₂ k₱ → x₅                      | p53_Mdm2 complex formation                    | k₁₀(x₁)(x₂) | 1155 × 10⁻⁷ sec⁻¹ | 3, 21, 27 |
| 11.  | x₂ k₁₁ → x₂                          | Mdm2 creation                                 | k₁₁(x₂)     | 825 × 10⁻⁴ sec⁻¹ | 3, 21, 27 |
| 12.  | x₅ k₁₂ → x₁ + x₂                     | Dissociation of p53_Mdm2 complex               | k₁₂(x₅)     | 1155 × 10⁻⁵ sec⁻¹ | 3, 21, 27 |
| 13.  | x₂ k₁₃ → φ                           | Mdm2 degradation                               | k₁₃(x₂)     | 433 × 10⁻⁴ sec⁻¹ | 3, 21, 27 |
| 14.  | φ k₁₄ → x₆                           | ROS formation                                  | k₁₄         | 1.0 × 10⁻² sec⁻¹ | 14, 21    |
| 15.  | x₆ k₁₅ → φ                           | Degradation of ROS                             | k₁₅(x₆)     | 2.0 × 10⁻² sec⁻¹ | 14, 21    |
| 16.  | x₆ k₁₆ → x₇                          | Initiation of DNA damage                       | k₁₆(x₆)     | 2.0 × 10⁻² sec⁻¹ | 14, 21    |
| 17.  | x₇ k₁₇ → φ                           | DNA repair                                     | k₁₇(x₇)     | 2.0 × 10⁻⁵ sec⁻¹ | 14, 21    |
| 18.  | x₇ k₁₈ → x₈ + x₇                     | Activation of ARF                              | k₁₈(x₇)     | 3.3 × 10⁻⁵ sec⁻¹ | 21, 24, 27 |
| 19.  | x₈ k₁₉ → φ                           | Degradation of ARF                             | k₁₉(x₈)     | 1.0 × 10⁻⁴ sec⁻¹ | 21, 24, 27 |
| 20.  | x₈ + x₂ k₂₀ → x₉                    | ARF_Mdm2 complex formation                    | k₂₀(x₈)(x₂) | 1.0 × 10⁻² sec⁻¹ | 21, 24, 27 |
| 21.  | x₉ k₂₁ → x₈                          | Dissociation of ARF_Mdm2 complex               | k₂₁(x₉)     | 1.0 × 10⁻⁵ sec⁻¹ | 21, 24, 27 |
| 22.  | x₁₀ k₂₂ → φ                           | Degradation of Micro RNA                       | k₂₂(x₁₀)   | 5.0 × 10⁻² sec⁻¹ | 16, 17    |

Table 2 - List of chemical reaction, Kinetic Laws and their rate constant