MATHEMATICAL BIOSCIENCES
AND ENGINEERING
Volume **12**, Number **6**, December **2015**

doi:10.3934/mbe.2015.12.1189

pp. **1189–1202**

SYNERGISTIC EFFECT OF BLOCKING CANCER CELL INVASION REVEALED BY COMPUTER SIMULATIONS

Kazuhisa Ichikawa

Division of Mathematical Oncology
Institute of Medical Science, University of Tokyo
4-6-1 Shirokanedai Minato-ku, Tokyo, 108-8639, Japan

Abstract. Invasion and metastasis are the main cause of death in cancer patients. The initial step of invasion is the degradation of extracellular matrix (ECM) by primary cancer cells in a tissue. Membranous metalloproteinase MT1-MMP and soluble metalloproteinase MMP-2 are thought to play an important role in the degradation of ECM. In the previous report, we found that the repetitive insertion of MT1-MMP to invadopodia was crucial for the effective degradation of ECM (Hoshino, D., et al., PLoS Comp. Biol., 2012, e1002479). However, the role of MMP-2 and the effect of inhibitors for these ECM-degrading proteases were still obscure. Here we investigated these two problems by using the same model as in the previous report. First we tested the effect of MMP-2 and found that while MT1-MMP played a major role in the degradation of ECM, MMP-2 played only a marginal effect on the degradation of ECM. Based on these findings, we next tested the effect of a putative inhibitor for MT1-MMP and found that such inhibitor was ineffective in blocking ECM degradation. Then we tested combined strategy including inhibitor for MT1-MMP, reduction of its turnover and its content in vesicles. A synergistic effect of combined strategy was observed in the decrease in the efficacy of ECM degradation. Our simulation study suggests the importance of combined strategy in blocking cancer invasion and metastasis.

1. **Introduction.** Invasion and metastasis are the main cause of death in cancer patients. If we control and prevent them, many cancer patients can survive. Thus finding therapeutic methods to block invasion and metastasis is inherently important. Invasion is the first step for cancer cells to metastasize, and many works has been published on the mechanisms of invasion. Normal cells reside in a tissue by being connected to each other and also to extracellular matrix (ECM). These prevent free movement of cells. An event required for malignant cancer cells to invade into tissue is the breaking of the cell-cell and cell-ECM connections. Cadherins and integrins are proteins of cell-cell and cell-ECM connection, respectively. These proteins are dysregulated in invading cancer cells. However for cells to move, their shapes should be changed. This is realized by the reorganization of actin cytoskeleton. This is another event for cancer cells to invade. In addition, ECM surrounding cells should be degraded for cells to move. This is also an important event. Thus the invasion is a phenomenon coordinated by these three events.

Among them, we have been focusing our simulation studies on the degradation of ECM. Malignant cancer cells constitutively express proteins degrading ECM

2010 *Mathematics Subject Classification.* 92C50.
Key words and phrases. Cancer, invasion, MT1-MMP, MMP-2, therapeutic targets.

[11, 7, 9]. These proteins are called MMPs (Matrix MetalloProteinases). More than 20 members of MMPs are reported [3], and we are interested in MT1-MMP (Membrane Type 1 MMP), because it is thought to play a central role in the ECM degradation at the initial step of invasion. MT1-MMP, which is a membrane protein, is concentrated at the tip of invadopodia, which are tiny protrusions of cells to degrade ECM [13]. The length and diameter of invadopodia are μm and sub-μm range, respectively. Experiments using cultured cancer cells have shown that the ECM degradation proceeded at the loci of invadopodia [1]. MT1-MMP made a tiny hole. The size of the hole becomes larger and larger, and finally cancer cells invade into a tissue thorough it [10]. Thus, one target of blockade of invasion is the inhibition of opening holes caused by MT1-MMP.

We have constructed a computational model for the regulation of MT1-MMP activity at invadopodia [4]. In this report, we tested potential methods to prevent ECM degradation by MT1-MMP. We reported the importance of the repetitive insertion of MT1-MMP to the membrane for the effective degradation of ECM in spatio-temporal (4D) model. We also reported the quite sharp activity of newly inserted MT1-MMP [12]. These findings have suggested us that a simple drug blocking the activity of MT1-MMP would not be effective for blocking ECM degradation by MT1-MMP. In this study, we tried a combined strategy of different blockade methods, and found that a combination of different methods was synergistically effective. In addition, we analyzed the activity of MT1-MMP and MMP-2 in the ECM degradation, and found that MT1-MMP played a major role in the ECM degradation, while MMP-2 possessed only a marginal effect.

2. **Model.** The model used in the present study was the same as before. Briefly, newly synthesized MT1-MMPs are transported to the invadopodial membrane by vesicle trafficking. Inserted MT1-MMPs were bound with ECM to degrade it, or inhibited by the TIMP-2, a soluble extracellular protein. MT1-MMP-bound TIMP-2 could bind proMMP-2, an inactive form of soluble ECM degrading protein, thus forming a ternary complex of MT1-MMP:TIMP-2:proMMP-2 [4]. If this ternary complex was bound with another TIMP-2-free MT1-MMP, it degraded proMMP-2 in the ternary complex leading to the generation of an active form of MMP-2. Activated MMP-2 degraded ECM together with MT1-MMP. According to our experiments, we assumed two independent pools on the membrane of MT1-MMP, pool X and pool D. These two pools possessed different kinetics in the turnover of MT1-MMP, which were measured by our FRAP experiments [4]. The turnover rates for pool X and pool D were $1/26$ and $1/259/s$, respectively. Pool D had a higher activity in the degradation of ECM with higher turnover rate and larger content of MT1-MMP than pool X ($69.9nM$ for pool D and $30.1nM$ for pool X, Figure 1A) [4].

Spatio-temporal model is constructed by dividing $5\mu m \times 5\mu m \times 3\mu m$ 3D cuboid shape into $51 \times 51 \times 1$ compartments of identical size (Figure 1B). Thus the size of each compartment was $0.0973\mu m \times 0.0973\mu m \times 3\mu m$. One surface of the shape was assumed to be the ventral surface of a cancer cell, and ECM was present all along the shape. Therefore, the shape modelled an extracellular space. TIMP-2, proMMP-2 and MMP-2 were assumed to diffuse at diffusion coefficient of $10^{-15}m^2/s$. This value was small in comparison to the coefficients for ordinary cytoplasmic soluble proteins ($10^{-10} - 10^{-12}m^2/s$), because extracellular space was assumed to be crowded with ECM proteins, and was present as a gel. This could lead to much

Fig.1

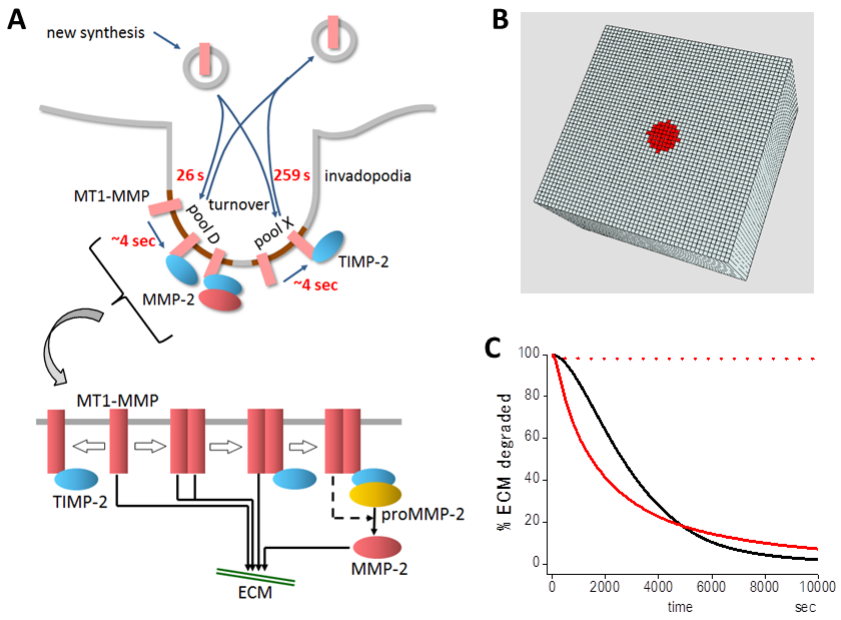

FIGURE 1. A model for the ECM degradation by MT1-MMP. A) MT1-MMP is inserted to pool D and pool X in the invadopodial membrane by different turnover rate of 26 and 259s. Inserted MT1-MMP is quickly inhibited with a time constant of 4s (top panel) as reported ([4]). Inserted MT1-MMP undergoes complex activation and inactivation modifications (bottom panel). B) 3D model of ECM degradation. The size of the cuboid shape was $5\mu m \times 5\mu m \times 3\mu m$ 3D, which were divided into $51 \times 51 \times 1$ compartments of identical size. Red region at the center indicates the compartment expressing MT1-MMP. C) Temporal simulation replicated the observed time course of ECM degradation (black curve). However, 4D simulation with the same parameter values did not replicate the observation showing virtually no ECM degradation (red dotted curve). If we introduce repetitive insertion of MT1-MMP, simulation result resembled the same time course as seen in the observation suggesting the importance of repetitive insertion of MT1-MMP.

reduced diffusion coefficient for proteins in a gel. Therefore, we employed the small value of the diffusion coefficient. We set 49 compartments at the center of the shape as an invadopodium (shown in red) whose diameter was about 0.9μm. Before the start of simulations, concentration of MT1-MMP at the invadipodial membrane was zero, and at $t = 0$, it was inserted to the membrane. These models were constructed using A-Cell software (`http://www.ims.u-tokyo.ac.jp/mathcancer/A-Cell/index_e.html`) [5, 6], which available from above URL. The detailed model, differential equations, and parameter values were the same as before [4, 12], and

can be downloaded from the same URL. We simulated a putative blocker for MT1-MMP (MTblock). In this case, we incorporated an additional reaction describing binding between MT1-MMP and MTblock as follows:

$$\mathrm{MT1-MMP} + \mathrm{MTblock} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \mathrm{MT1-MMP : MTblock}. \tag{1}$$

k_f, k_b, and initial concentration of MT-block were $1.53\times10^5/M/s$, $5.85\times10^{-4}/s$, and $7\mu M$, respectively. These values were modified from a known drag imatinib. The concentration of MTblock was set relatively large aimed at acquiring an effective blockade of active MT1-MMP. Differential equations derived from (1) were added to the existing differential equation for MT1-MMP as follows (Cf. Appendix):

$$\begin{aligned}\frac{\partial[MT1MMP]}{\partial} &= f(MT1MMP, TIMP2, \ldots) + D\nabla^2[MT1MMP] \\ &\quad -k_f[MTblock][MT1MMP] + k_b[MT1MMP : MTblock]\end{aligned} \tag{2}$$

The first and second terms on the right hand side are other reactions than scheme (1) and the diffusion of MT1-MMP, respectively. The third and fourth terms are for the scheme (1). Considerable part of model parameters was extracted from experimental reports [4, 12].

3. **Results.** First we show comparisons between the temporal and spatio-temporal (4D) simulation results for ECM degradation. While in the temporal simulation without space, ECM was almost completely degraded at $3h$ (black continuous curve in Figure 1C), which was consistent with the reported observation [1], it was degraded only a few portion of ECM in the spatio-temporal (4D) simulation (1%, dotted red curve in Figure 1C). This situation was greatly changed when MT1-MMP was inserted repetitively at shorter intervals than $75s$. This protocol led to a consistent result with reported observations (red continuous curve in Figure 1C). Thus the repetitive insertion of MT1-MMP was required for the appreciable degradation of ECM as shown in the previous report [4, 12]. This prediction of the simulation was experimentally tested by applying various blockers of vesicle trafficking. We found that these blockers inhibited ECM degradation almost completely [4]. By the careful analysis of simulation results, we found a sharp transient activity with half width of about 4 sec just after the insertion of MT1-MMP to the membrane [12]. These results led us to conclude that the activity of MT1-MMP at invadopodia is dynamically regulated with unexpectedly fast kinetics (Figure 1A).

Next, we tried to search the relative contribution of ECM degradation by MT1-MMP and MMP-2, since ECM degradation by MMP-2 required MT1-MMP activity as shown above, and this raised a possibility that ECM degradation by MMP-2 would be less effective than by MT1-MMP. Surprisingly there was no change in the time course of ECM degradation in the presence or absence of MMP-2 (Figure 2A). If we plotted time courses of the complex MT1-MMP:ECM and MMP-2:ECM, we found the appreciable formation of MMP-2:ECM only at the end of the ECM degradation (Figure 2B). However, the maximum relative amount of MMP-2:ECM complex was less than 1% to that of MT1-MMP:ECM complex. This raised a possibility that there was a rivalry between ECM and TIMP-2:proMMP-2 in the binding to MT1-MMP, and MT1-MMP preferentially bound to ECM (Figure 2C). If this were the case, reduction in the binding kinetics between MT1-MMP and ECM and increase in the ECM-degrading kinetics on MMP-2 would increase the

Fig.2

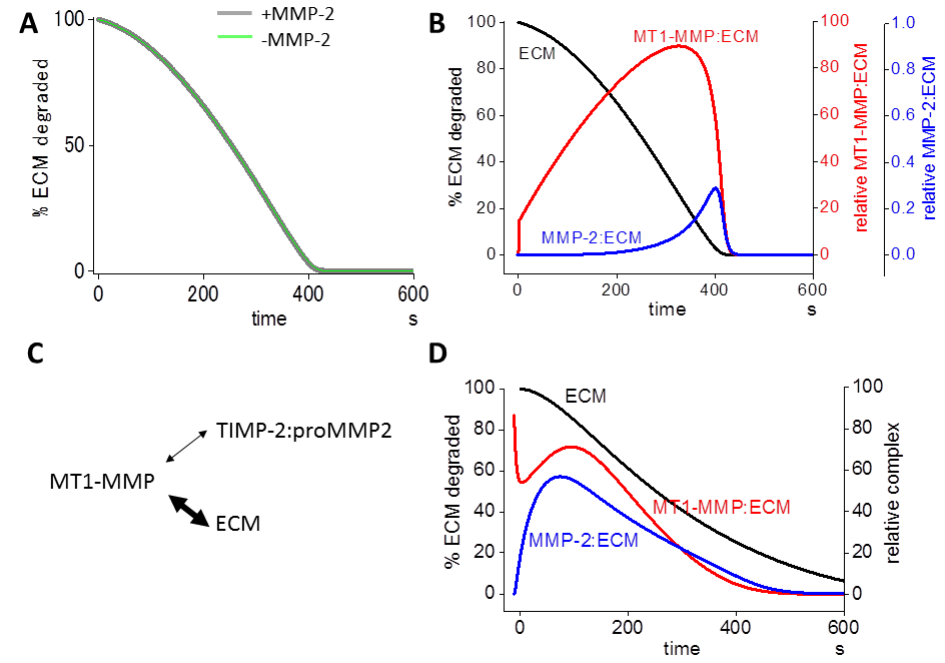

FIGURE 2. The contribution of MMP-2 in the degradation of ECM. A) There was virtually no difference in the ECM degradation between in the presence and in the absence of MMP-2. B) MMP-2 formed a complex with ECM with only a small amount at the end of the ECM degradation. Thus the MMP-2 contribution to the ECM degradation was small. C) There is a rivalry between ECM and TIMP-2:proMMP-2 in the binding with MT1-MMP, and MT1-MMP preferentially binds to ECM. D) If the affinity of MT1-MMP to ECM was decreased one-100$^{\text{th}}$ and the catalytic activity of MMP-2 to degrade ECM was increased 100-fold, MMP-2 contributed ECM degradation appreciably.

contribution of MMP-2 in the degradation of ECM. This was the case as shown in Figure 2D, where MMP-2:ECM complex was formed at an earlier stage of ECM degradation, and MMP-2 resembled a considerable contribution for the degradation of ECM. In this simulation, the binding and dissociation rate constants between MT1-MMP and ECM were 0.1- and 10-fold, respectively, and the ECM degrading catalytic activity of MMP-2 was increased 100-fold. Thus in our control condition, MT1-MMP played a major role in the degradation of ECM. It was reported that MT1-MMP and MMP-2 possessed different affinity to different component of ECM [8, 2], and these two MMPs would play different roles in the actual environment of invasion.

Then the next question was how we could block ECM degradation. As shown above, MT1-MMP played a major role, we focused on the blockade of MT1-MMP.

Our simulation showed a quick turnover of MT1-MMP at the invadopodial membrane [4]. If this was the case, it would be not easy to block the activity of MT1-MMP by its inhibitor, which is a traditional strategy. In fact, we found only a small effect with the addition of such inhibitor. In the simulation, we added a reaction scheme for a putative MT1-MMP blocker, MTblock, as shown in reaction scheme (1). We employed time to half-degradation of ECM (t_H) as a measure of the effectiveness of ECM degradation. MTblock showed only a marginal effect on the blockade of ECM degradation (Figure 3A).

Then we tried to test other strategies to block ECM degradation. There were at least two additional methods to block ECM degradation, reductions in vesicular content of MT1-MMP and in the turnover rate of MT1-MMP-containing vesicles (Figure 3B). We tested these possibilities by changing rate constants in our differential equations (Appendix). The Simulation results are shown in Figure 3C together with those of MTblock. At TIMP-2 concentration of $180nM$, there was only 26.7-fold increase in the t_H by one-50th reduction in the vesicular content (dark blue line in Figure 3C). There was only 8.8-fold increase in the t_H by one-50th reduction in the turnover rate (light blue line). There was only 24.9-hold increase in t_H by 50-hold increase in the concentration of MTblock from 7μM (blue line). Thus, there was only a small effect on the reduction in t_H by a single treatment among three possible methods.

Next, we tested the combined effect of three different methods. There was 901-fold increase in the increase in t_H by the one-50th reduction both in the vesicular content and the turnover rate (magenta line in Figure 3D). If these two processes are independent, combination of the two processes would yield 235-fold increase (26.7×8.8). However, the resulting increase was higher than this, suggesting an existence of synergistic effect. If we add MTblock to this combined method, there was 1893-fold increase in t_H (red line in Figure 3D). In this case, however, fold increase in t_H was smaller than the expected value (5851-fold$= 8.8 \times 24.9 \times 26.7$), which was the linear combination of three methods. Thus, we have found a synergistic effect in the increase of t_H if we selected an appropriate combination of methods showing a potential effect of combined inhibition strategy.

4. **Discussion.** Our simulations suggested a potential synergistic effect of combined strategy in the blockade of the activity of MT1-MMP. If we want to develop a drug blocking vesicle trafficking only for those containing MT1-MMP, a drug should discriminate vesicles containing MT1-MMP from those not containing it. The development of such a drug would be difficult. However, our simulation results suggested that a simple blocker of MT1-MMP activity was not enough to block the ECM degradation largely because of the abundance of ECM in comparison to the drug and the higher affinity of MT1-MMP to TIMP-2 than to the drug. If we consider the effectiveness in the blockade of MT1-MMP activity by the combined strategy, the development of such a drug will be the next important target. Simulation results showed the synergistic and non-synergistic effect on the blockade of the activity of MT1-MMP (Figure 3). These are because of the nonlinear behavior of the model. It is not easy to predict synergistic or non-synergistic behavior only by mathematical analyses. On the contrary, simulations can predict such behaviors. However, it is not easy both for simulation and mathematical analysis alone to find a reason for the non-linear effect. Thus, it is desired to explore a method to analyze non-linear effect by a collaboration of simulation and mathematical analysis.

Fig.3

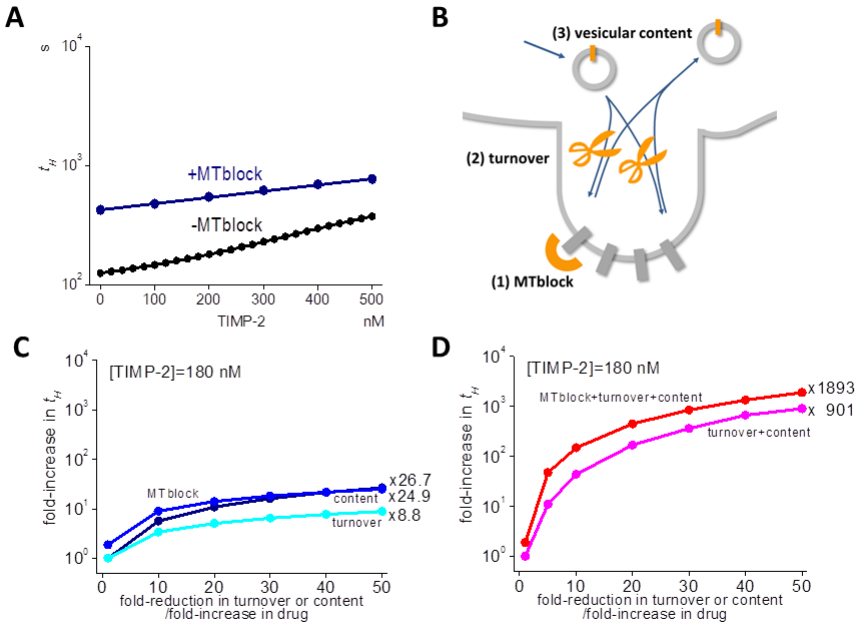

FIGURE 3. Synergistic effect in blocking ECM degradation by the reduction in the vesicular content and its turnover rate. A) A putative blocker of MT1-MMP (MTblock) showed only marginal effect on the blockade of ECM degradation. B) Three methods to block the activity of MT1-MMP. C) Application of one of three methods showed only a small effect. D) There was a potentially synergistic effect in the blockade of the MT1-MMP activity by the combination of two methods.

Appendix. Differential equations for the reaction including a putative inhibitor MTblock. Since we found two independent pools for MT1-MMP with different kinetics in an invadopodium ([4]), we distinguish MT1-MMP in different pools X and D by M14x and M14D, respectively.

/* MT1-MMP(M14x and M14D), TIMP2(T2), and MMP2(M2) ternary complex for pool X */

$$
\begin{aligned}
d[M14x]/dt = &-kT2 * [M14x] * [T2] + kT2\_ * [M14x.T2] \\
&- kM14 * [M14x] * [M14x] + kM14\_ * [M14x.M14x] \\
&- kM14 * [M14x] * [M14x] + kM14\_ * [M14x.M14x] \\
&- kM14 * [M14x.T2] * [M14x] + kM14\_ * [M14x.M14x.T2] \\
&- kM14 * [M14x.T2.M2] * [M14x] + kM14\_ * [M14x.M14x.T2.M2] \\
&- kT2 * [M14x] * [T2.M2] + kT2\_ * [M14x.T2.M2] \\
&- CX * [M14x]/[M14xGT] + kX * [MF] - kfn11 * [M14x] * [fn] \\
&+ kfn11\_ * [M14x.fn] + kfn11p * [M14x.fn]
\end{aligned}
$$

$$
\begin{aligned}
&\quad - k\_f[MTblock][MT1MMP] + k\_b[MT1MMP : MTblock]\\
d[T2]/dt &= -kT2 * [M14x] * [T2] + kT2\_ * [M14x.T2] - kT2 * [M14x.M14x] * [T2]\\
&\quad + kT2\_ * [M14x.M14x.T2] - kT2 * [M14x.M14x.T2] * [T2]\\
&\quad + kT2\_ * [M14x.T2.M14x.T2] - kT2 * [M14x.M14x.T2.M2] * [T2]\\
&\quad + kT2\_ * [M14x.T2.M14x.T2.M2] - kM2 * [T2] * [M2]\\
&\quad - kT2 * [M14D] * [T2] + kT2\_ * [M14D.T2] - kT2 * [M14D.M14D] * [T2]\\
&\quad + kT2\_ * [M14D.M14D.T2] - kT2 * [M14D.M14D.T2] * [T2]\\
&\quad + kT2\_ * [M14D.T2.M14D.T2] - kT2 * [M14D.M14D.T2.M2] * [T2]\\
&\quad + kT2\_ * [M14D.T2.M14D.T2.M2] - kM2 * [T2] * [M2]\\
&\quad + CX * ([M14x.T2] + [M14x.T2.M2] + [M14x.M14x.T2]\\
&\quad + [M14x.M14x.T2.M2] + 2 * ([M14x.T2.M14x.T2]\\
&\quad + [M14x.T2.M14x.T2.M2] + [M14x.T2.M2.M14x.T2.M2]))/[M14xGT]\\
&\quad + kD * [M14D.M14D.T2] + kD * [M14D.M14D.T2.M2]\\
&\quad + kD * [M14D.T2.M2] + kD * [M14D.T2.M14D.T2]\\
&\quad + kD * [M14D.T2.M14D.T2] + kD * [M14D.T2.M14D.T2.M2]\\
&\quad + kD * [M14D.T2.M14D.T2.M2] + kD * [M14D.T2]\\
&\quad + kD * [M14D.T2.M2.M14D.T2.M2]\\
&\quad + kD * [M14D.T2.M2.M14D.T2.M2] - kM2aT * [M2act] * [T2]\\
d[M14x.T2]/dt &= kT2 * [M14x] * [T2] - kT2\_ * [M14x.T2]\\
&\quad - kM14 * [M14x.T2] * [M14x] + kM14\_ * [M14x.M14x.T2]\\
&\quad - kM14 * [M14x.T2] * [M14x.T2] + kM14\_ * [M14x.T2.M14x.T2]\\
&\quad - kM14 * [M14x.T2] * [M14x.T2] + kM14\_ * [M14x.T2.M14x.T2]\\
&\quad - kM14 * [M14x.T2.M2] * [M14x.T2]\\
&\quad + kM14\_ * [M14x.T2.M14x.T2.M2] - kM2 * [M14x.T2] * [M2]\\
&\quad - CX * [M14x.T2]/[M14xGT]\\
d[M14x.M14x]/dt &= kM14 * [M14x] * [M14x] - kM14\_ * [M14x.M14x]\\
&\quad - kT2 * [M14x.M14x] * [T2] + kT2\_ * [M14x.M14x.T2]\\
&\quad - kT2 * [M14x.M14x] * [T2.M2] + kT2\_ * [M14x.M14x.T2.M2]\\
&\quad - CX * [M14x.M14x]/[M14xGT]\\
&\quad - kfn12 * [M14x.M14x] * [fn] + kfn12\_ * [M14x.M14x.fn]\\
&\quad + kfn11p * [M14x.M14x.fn]\\
d[M14x.M14x.T2]/dt &= kT2 * [M14x.M14x] * [T2] - kT2\_ * [M14x.M14x.T2]\\
&\quad + kM14 * [M14x.T2] * [M14x] - kM14\_ * [M14x.M14x.T2]\\
&\quad - kT2 * [M14x.M14x.T2] * [T2] + kT2\_ * [M14x.T2.M14x.T2]\\
&\quad - kT2 * [M14x.M14x.T2] * [T2.M2] + kT2\_ * [M14x.T2.M14x.T2.M2]\\
&\quad - kM2 * [M14x.M14x.T2] * [M2] - CX * [M14x.M14x.T2]/[M14xGT]\\
&\quad + kM2act * [M14x.M14x.T2.M2] - kfn11 * [M14x.M14x.T2] * [fn]\\
&\quad + kfn11\_ * [M14x.M14x.T2.fn] + kfn11p * [M14x.M14x.T2.fn]
\end{aligned}
$$

$$
\begin{aligned}
d[M14x.T2.M2]/dt &= -kM14 * [M14x.T2.M2] * [M14x] \\
&\quad + kM14\_ * [M14x.M14x.T2.M2] - kM14 * [M14x.T2.M2] * [M14x.T2.M2] \\
&\quad + kM14\_ * [M14x.T2.M2.M14x.T2.M2] \\
&\quad - kM14 * [M14x.T2.M2] * [M14x.T2.M2] \\
&\quad + kM14\_ * [M14x.T2.M2.M14x.T2.M2] + kT2 * [M14x] * [T2.M2] \\
&\quad - kT2\_ * [M14x.T2.M2] - kM14 * [M14x.T2.M2] * [M14x.T2] \\
&\quad + kM14\_ * [M14x.T2.M14x.T2.M2] + kM2 * [M14x.T2] * [M2] \\
&\quad - CX * [M14x.T2.M2]/[M14xGT] \\
d[M14x.M14x.T2.M2]/dt &= kM14 * [M14x.T2.M2] * [M14x] \\
&\quad - kM14\_ * [M14x.M14x.T2.M2] - kT2 * [M14x.M14x.T2.M2] * [T2] \\
&\quad + kT2\_ * [M14x.T2.M14x.T2.M2] + kT2 * [M14x.M14x] * [T2.M2] \\
&\quad - kT2\_ * [M14x.M14x.T2.M2] - kT2 * [M14x.M14x.T2.M2] * [T2.M2] \\
&\quad + kT2\_ * [M14x.T2.M2.M14x.T2.M2] + kM2 * [M14x.M14x.T2] * [M2] \\
&\quad - CX * [M14x.M14x.T2.M2]/[M14xGT] - kM2act * [M14x.M14x.T2.M2] \\
&\quad - kfn11 * [M14x.M14x.T2.M2] * [fn] + kfn11\_ * [M14x.M14x.T2.M2.fn] \\
&\quad + kfn11p * [M14x.M14x.T2.M2.fn] \\
d[M14x.T2.M14x.T2]/dt &= kT2 * [M14x.M14x.T2] * [T2] \\
&\quad - kT2\_ * [M14x.T2.M14x.T2] + kM14 * [M14x.T2] * [M14x.T2] \\
&\quad - kM14\_ * [M14x.T2.M14x.T2] - kM2 * [M14x.T2.M14x.T2] * [M2] \\
&\quad - CX * [M14x.T2.M14x.T2]/[M14xGT] \\
d[M14x.T2.M14x.T2.M2]/dt &= kT2 * [M14x.M14x.T2.M2] * [T2] \\
&\quad - kT2\_ * [M14x.T2.M14x.T2.M2] + kT2 * [M14x.M14x.T2] * [T2.M2] \\
&\quad - kT2\_ * [M14x.T2.M14x.T2.M2] + kM14 * [M14x.T2.M2] * [M14x.T2] \\
&\quad - kM14\_ * [M14x.T2.M14x.T2.M2] + kM2 * [M14x.T2.M14x.T2] * [M2] \\
&\quad - kM2 * [M14x.T2.M14x.T2.M2] * [M2] \\
&\quad - CX * [M14x.T2.M14x.T2.M2]/[M14xGT] \\
d[M14x.T2.M2.M14x.T2.M2]/dt &= kM14 * [M14x.T2.M2] * [M14x.T2.M2] \\
&\quad - kM14\_ * [M14x.T2.M2.M14x.T2.M2] \\
&\quad + kT2 * [M14x.M14x.T2.M2] * [T2.M2] \\
&\quad - kT2\_ * [M14x.T2.M2.M14x.T2.M2] \\
&\quad + kM2 * [M14x.T2.M14x.T2.M2] * [M2] \\
&\quad - CX * [M14x.T2.M2.M14x.T2.M2]/[M14xGT] \\
d[T2.M2]/dt &= -kT2 * [M14x] * [T2.M2] + kT2\_ * [M14x.T2.M2] \\
&\quad - kT2 * [M14x.M14x] * [T2.M2] + kT2\_ * [M14x.M14x.T2.M2] \\
&\quad - kT2 * [M14x.M14x.T2] * [T2.M2] + kT2\_ * [M14x.T2.M14x.T2.M2] \\
&\quad - kT2 * [M14x.M14x.T2.M2] * [T2.M2] \\
&\quad + kT2\_ * [M14x.T2.M2.M14x.T2.M2] + kM2 * [T2] * [M2] \\
&\quad - kT2 * [M14D] * [T2.M2] + kT2\_ * [M14D.T2.M2]
\end{aligned}
$$

$$
\begin{aligned}
&- kT2 * [M14D.M14D] * [T2.M2] + kT2\_ * [M14D.M14D.T2.M2] \\
&- kT2 * [M14D.M14D.T2] * [T2.M2] + kT2\_ * [M14D.T2.M14D.T2.M2] \\
&- kT2 * [M14D.M14D.T2.M2] * [T2.M2] \\
&+ kT2\_ * [M14D.T2.M2.M14D.T2.M2] + kM2 * [T2] * [M2] \\
d[M2]/dt = &- kM2 * [T2] * [M2] - kM2 * [M14x.T2] * [M2] \\
&- kM2 * [M14x.M14x.T2] * [M2] - kM2 * [M14x.T2.M14x.T2] * [M2] \\
&- kM2 * [M14x.T2.M14x.T2.M2] * [M2] - kM2 * [T2] * [M2] \\
&- kM2 * [M14D.T2] * [M2] - kM2 * [M14D.M14D.T2] * [M2] \\
&- kM2 * [M14D.T2.M14D.T2] * [M2] \\
&- kM2 * [M14D.T2.M14D.T2.M2] * [M2] + CX * ([M14x.T2.M2] \\
&+ [M14x.M14x.T2.M2] + [M14x.T2.M14x.T2.M2] \\
&+ 2 * [M14x.T2.M2.M14x.T2.M2])/[M14xGT] \\
&+ kD * [M14D.M14D.T2.M2] + kD * [M14D.T2.M2] \\
&+ kD * [M14D.T2.M14D.T2.M2] + kD * [M14D.T2.M2.M14D.T2.M2] \\
&+ kD * [M14D.T2.M2.M14D.T2.M2] - k1 * [M2] + k1 * [M2 \\
\text{/* MT1-MMP, TIMP2, and MMP2 ternary complex for pool D */} & \\
d[M14D]/dt = &- kT2 * [M14D] * [T2] + kT2\_ * [M14D.T2] \\
&- kM14 * [M14D] * [M14D] + kM14\_ * [M14D.M14D] \\
&- kM14 * [M14D] * [M14D] + kM14\_ * [M14D.M14D] \\
&- kM14 * [M14D.T2] * [M14D] + kM14\_ * [M14D.M14D.T2] \\
&- kM14 * [M14D.T2.M2] * [M14D] + kM14\_ * [M14D.M14D.T2.M2] \\
&- kT2 * [M14D] * [T2.M2] + kT2\_ * [M14D.T2.M2] + CD * [Cpd] \\
&- kD * [M14D] - kfn11 * [M14D] * [fn] + kfn11\_ * [M14D.fn] \\
&+ kfn11p * [M14D.fn] \\
d[M14D.T2]/dt = &\ kT2 * [M14D] * [T2] - kT2\_ * [M14D.T2] \\
&- kM14 * [M14D.T2] * [M14D] + kM14\_ * [M14D.M14D.T2] \\
&- kM14 * [M14D.T2] * [M14D.T2] + kM14\_ * [M14D.T2.M14D.T2] \\
&- kM14 * [M14D.T2] * [M14D.T2] + kM14\_ * [M14D.T2.M14D.T2] \\
&- kM14 * [M14D.T2.M2] * [M14D.T2] + kM14\_ * [M14D.T2.M14D.T2.M2] \\
&- kM2 * [M14D.T2] * [M2] - kD * [M14D.T2] \\
d[M14D.M14D]/dt = &\ kM14 * [M14D] * [M14D] - kM14\_ * [M14D.M14D] \\
&- kT2 * [M14D.M14D] * [T2] + kT2\_ * [M14D.M14D.T2] \\
&- kT2 * [M14D.M14D] * [T2.M2] + kT2\_ * [M14D.M14D.T2.M2] \\
&- kD * [M14D.M14D] - kfn12 * [M14D.M14D] * [fn] \\
&+ kfn12\_ * [M14D.M14D.fn] + kfn11p * [M14D.M14D.fn] \\
d[M14D.M14D.T2]/dt = &\ kT2 * [M14D.M14D] * [T2] - kT2\_ * [M14D.M14D.T2] \\
&+ kM14 * [M14D.T2] * [M14D] - kM14\_ * [M14D.M14D.T2] \\
&- kT2 * [M14D.M14D.T2] * [T2] + kT2\_ * [M14D.T2.M14D.T2]
\end{aligned}
$$

$$
\begin{aligned}
&- kT2 * [M14D.M14D.T2] * [T2.M2] + kT2\_ * [M14D.T2.M14D.T2.M2] \\
&- kM2 * [M14D.M14D.T2] * [M2] - kD * [M14D.M14D.T2] \\
&+ kM2act * [M14D.M14D.T2.M2] - kfn11 * [M14D.M14D.T2] * [fn] \\
&+ kfn11\_ * [M14D.M14D.T2.fn] + kfn11p * [M14D.M14D.T2.fn] \\
d[M14D.T2.M2]/dt &= -kM14 * [M14D.T2.M2] * [M14D] \\
&+ kM14\_ * [M14D.M14D.T2.M2] - kM14 * [M14D.T2.M2] * [M14D.T2.M2] \\
&+ kM14\_ * [M14D.T2.M2.M14D.T2.M2] \\
&- kM14 * [M14D.T2.M2] * [M14D.T2.M2] \\
&+ kM14\_ * [M14D.T2.M2.M14D.T2.M2] + kT2 * [M14D] * [T2.M2] \\
&- kT2\_ * [M14D.T2.M2] - kM14 * [M14D.T2.M2] * [M14D.T2] \\
&+ kM14\_ * [M14D.T2.M14D.T2.M2] + kM2 * [M14D.T2] * [M2] \\
&- kD * [M14D.T2.M2] \\
d[M14D.M14D.T2.M2]/dt &= kM14 * [M14D.T2.M2] * [M14D] \\
&- kM14\_ * [M14D.M14D.T2.M2] - kT2 * [M14D.M14D.T2.M2] * [T2] \\
&+ kT2\_ * [M14D.T2.M14D.T2.M2] + kT2 * [M14D.M14D] * [T2.M2] \\
&- kT2\_ * [M14D.M14D.T2.M2] - kT2 * [M14D.M14D.T2.M2] * [T2.M2] \\
&+ kT2\_ * [M14D.T2.M2.M14D.T2.M2] + kM2 * [M14D.M14D.T2] * [M2] \\
&- kD * [M14D.M14D.T2.M2] - kM2act * [M14D.M14D.T2.M2] \\
&- kfn11 * [M14D.M14D.T2.M2] * [fn] + kfn11\_ * [M14D.M14D.T2.M2.fn] \\
&+ kfn11p * [M14D.M14D.T2.M2.fn] \\
d[M14D.T2.M14D.T2]/dt &= kT2 * [M14D.M14D.T2] * [T2] \\
&- kT2\_ * [M14D.T2.M14D.T2] + kM14 * [M14D.T2] * [M14D.T2] \\
&- kM14\_ * [M14D.T2.M14D.T2] - kM2 * [M14D.T2.M14D.T2] * [M2] \\
&- kD * [M14D.T2.M14D.T2] \\
d[M14D.T2.M14D.T2.M2]/dt &= kT2 * [M14D.M14D.T2.M2] * [T2] \\
&- kT2\_ * [M14D.T2.M14D.T2.M2] + kT2 * [M14D.M14D.T2] * [T2.M2] \\
&- kT2\_ * [M14D.T2.M14D.T2.M2] + kM14 * [M14D.T2.M2] * [M14D.T2] \\
&- kM14\_ * [M14D.T2.M14D.T2.M2] + kM2 * [M14D.T2.M14D.T2] * [M2] \\
&- kM2 * [M14D.T2.M14D.T2.M2] * [M2] - kD * [M14D.T2.M14D.T2.M2] \\
d[M14D.T2.M2.M14D.T2.M2]/dt &= kM14 * [M14D.T2.M2] * [M14D.T2.M2] \\
&- kM14\_ * [M14D.T2.M2.M14D.T2.M2] \\
&+ kT2 * [M14D.M14D.T2.M2] * [T2.M2] \\
&- kT2\_ * [M14D.T2.M2.M14D.T2.M2] \\
&+ kM2 * [M14D.T2.M14D.T2.M2] * [M2] \\
&- kD * [M14D.T2.M2.M14D.T2.M2] \\
&/* \text{ total [M14D] which is not bound with ECM } */ \\
[M14Dt] &= [M14D] + [M14D.T2] + [M14D.T2.M2] + 2 * [M14D.M14D] \\
&+ 2 * [M14D.M14D.T2] + 2 * [M14D.M14D.T2.M2]
\end{aligned}
$$

$$+ 2 * [M14D.T2.M14D.T2] + 2 * [M14D.T2.M2.M14D.T2.M2]$$
$$+ 2 * [M14D.T2.M14D.T2.M2]$$

/* insertion of MT1-MMP to PX */

$$d[MF]/dt = -kX * [MF]$$

/* internalization of MT1-MMP and recycling of TIMP2 and MMP2 for pool D*/

$$d[M14Di]/dt = kD * [M14D] + kD * [M14D.T2.M2] + kD * [M14D.T2]$$
$$d[M14Di.M14Di]/dt = kD * [M14D.M14D] + kD * [M14D.M14D.T2]$$
$$+ kD * [M14D.M14D.T2.M2] + kD * [M14D.T2.M14D.T2]$$
$$+ kD * [M14D.T2.M14D.T2.M2] + kD * [M14D.T2.M2.M14D.T2.M2]$$

/* MMP2 activation */

$$d[M2act]/dt = kM2act * [M14x.M14x.T2.M2] + kM2act * [M14D.M14D.T2.M2]$$
$$- kM2aT * [M2act] * [T2] - k1 * [M2act] + k1 * [M2act]$$
$$- kfn2 * [M2act] * [fn] + kfn2\_ * [M2act.fn] + kfn2p * [M2act.fn]$$

/* MMP2 inactivation */

$$d[M2act.T2]/dt = kM2aT * [M2act] * [T2]$$

/* ECM degradation by MT1-MMP */

$$d[fn]/dt = -kfn11 * [M14x] * [fn] + kfn11\_ * [M14x.fn]$$
$$- kfn12 * [M14x.M14x] * [fn] + kfn12\_ * [M14x.M14x.fn]$$
$$- kfn11 * [M14x.M14x.T2] * [fn] + kfn11\_ * [M14x.M14x.T2.fn]$$
$$- kfn11 * [M14x.M14x.T2.M2] * [fn] + kfn11\_ * [M14x.M14x.T2.M2.fn]$$
$$- kfn11 * [M14D] * [fn] + kfn11\_ * [M14D.fn]$$
$$- kfn12 * [M14D.M14D] * [fn] + kfn12\_ * [M14D.M14D.fn]$$
$$- kfn11 * [M14D.M14D.T2] * [fn] + kfn11\_ * [M14D.M14D.T2.fn]$$
$$- kfn11 * [M14D.M14D.T2.M2] * [fn] + kfn11\_ * [M14D.M14D.T2.M2.fn]$$
$$- kfn11 * [M14x.M14x.fn] * [fn] + kfn11\_ * [fn.M14x.M14x.fn]$$
$$- kfn11 * [M14D.M14D.fn] * [fn] + kfn11\_ * [fn.M14D.M14D.fn]$$
$$- kfn2 * [M2act] * [fn] + kfn2\_ * [M2act.fn]$$
$$d[M14x.fn]/dt = kfn11 * [M14x] * [fn]$$
$$- kfn11\_ * [M14x.fn] - kfn11p * [M14x.fn]$$
$$d[fnd]/dt = kfn11p * [M14x.fn] + kfn11p * [M14x.M14x.fn]$$
$$+ kfn11p * [M14x.M14x.T2.fn] + kfn11p * [M14x.M14x.T2.M2.fn]$$
$$+ kfn11p * [M14D.fn] + kfn11p * [M14D.M14D.fn]$$
$$+ kfn11p * [M14D.M14D.T2.fn] + kfn11p * [M14D.M14D.T2.M2.fn]$$
$$+ kfn12p * [fn.M14x.M14x.fn] + kfn12p * [fn.M14D.M14D.fn]$$
$$+ kfn2p * [M2act.fn]$$
$$d[M14x.M14x.fn]/dt = kfn12 * [M14x.M14x] * [fn]$$
$$- kfn12\_ * [M14x.M14x.fn] - kfn11p * [M14x.M14x.fn]$$
$$- kfn11 * [M14x.M14x.fn] * [fn] + kfn11\_ * [fn.M14x.M14x.fn]$$

$$+ kfn12p * [fn.M14x.M14x.fn]$$
$$d[M14x.M14x.T2.fn]/dt = kfn11 * [M14x.M14x.T2] * [fn]$$
$$- kfn11\_ * [M14x.M14x.T2.fn] - kfn11p * [M14x.M14x.T2.fn]$$
$$d[M14x.M14x.T2.M2.fn]/dt = kfn11 * [M14x.M14x.T2.M2] * [fn]$$
$$- kfn11\_ * [M14x.M14x.T2.M2.fn] - kfn11p * [M14x.M14x.T2.M2.fn]$$
$$d[M14D.fn]/dt = kfn11 * [M14D] * [fn]$$
$$- kfn11\_ * [M14D.fn] - kfn11p * [M14D.fn]$$
$$d[M14D.M14D.fn]/dt = kfn12 * [M14D.M14D] * [fn]$$
$$- kfn12\_ * [M14D.M14D.fn] - kfn11p * [M14D.M14D.fn]$$
$$- kfn11 * [M14D.M14D.fn] * [fn] + kfn11\_ * [fn.M14D.M14D.fn]$$
$$+ kfn12p * [fn.M14D.M14D.fn]$$
$$d[M14D.M14D.T2.fn]/dt = kfn11 * [M14D.M14D.T2] * [fn]$$
$$- kfn11\_ * [M14D.M14D.T2.fn] - kfn11p * [M14D.M14D.T2.fn]$$
$$d[M14D.M14D.T2.M2.fn]/dt = kfn11 * [M14D.M14D.T2.M2] * [fn]$$
$$- kfn11\_ * [M14D.M14D.T2.M2.fn] - kfn11p * [M14D.M14D.T2.M2.fn]$$
$$d[fn.M14x.M14x.fn]/dt = kfn11 * [M14x.M14x.fn] * [fn]$$
$$- kfn11\_ * [fn.M14x.M14x.fn] - kfn12p * [fn.M14x.M14x.fn]$$
$$d[fn.M14D.M14D.fn]/dt = kfn11 * [M14D.M14D.fn] * [fn]$$
$$- kfn11\_ * [fn.M14D.M14D.fn] - kfn12p * [fn.M14D.M14D.fn]$$
/* ECM degradation by MMP-2 */
$$d[M2act.fn]/dt = kfn2 * [M2act] * [fn]$$
$$- kfn2\_ * [M2act.fn] - kfn2p * [M2act.fn]$$

REFERENCES

[1] V. V. Artym, Y. Zhang, F. Seillier-Moiseiwitsch, K. M. Yamada and S. C. Mueller, Dynamic interactions of cortactin and membrane type 1 matrix metalloproteinase at invadopodia: defining the stages of invadopodia formation and function, *Cancer Res*, **66** (2006), 3034–3043.

[2] H. F. Bigg, C. J. Morrison, G. S. Butler, M. A. Bogoyevitch and Z. Wang, et al., Tissue inhibitor of metalloproteinases-4 inhibits but does not support the activation of gelatinase A via efficient inhibition of membrane type 1-matrix metalloproteinase, *Cancer Res*, **61** (2001), 3610–3618.

[3] M. Egeblad and Z. Werb, New functions for the matrix metalloproteinases in cancer progression, *NatRevCancer*, **2** (2002), 161–174.

[4] D. Hoshino, N. Koshikawa, T. Suzuki, V. Quaranta and A. M. Weaver, et al., Establishment and validation of computational model for MT1-MMP dependent ECM degradation and intervention strategies, *PLoS Comput Biol*, **8** (2012), e1002479.

[5] K. Ichikawa, A-Cell: graphical user interface for the construction of biochemical reaction models, *Bioinformatics*, **17** (2001), 483–484.

[6] K. Ichikawa, A modeling environment with three-dimensional morphology, A-Cell-3D, and Ca2+ dynamics in a spine, *Neuroinformatics*, **3** (2005), 49–64.

[7] E. Maquoi, D. Assent, J. Detilleux, C. Pequeux and J. M. Foidart, et al., MT1-MMP protects breast carcinoma cells against type I collagen-induced apoptosis, *Oncogene*, **31** (2012), 480–493.

[8] H. Nagase, R. Visse and G. Murphy, Structure and function of matrix metalloproteinases and TIMPs, *Cardiovasc Res*, **69** (2006), 562–573.

[9] T. Nonaka, K. Nishibashi, Y. Itoh, I. Yana and M. Seiki, Competitive disruption of the tumor-promoting function of membrane type 1 matrix metalloproteinase/matrix metalloproteinase-14 in vivo, *MolCancer Ther*, **4** (2005), 1157–1166.
[10] M. Schoumacher, R. D. Goldman, D. Louvard and D. M. Vignjevic, Actin, microtubules, and vimentin intermediate filaments cooperate for elongation of invadopodia, *J Cell Biol*, **189** (2010), 541–556.
[11] K. Taniwaki, H. Fukamachi, K. Komori, Y. Ohtake and T. Nonaka, et al., Stroma-derived matrix metalloproteinase (MMP)-2 promotes membrane type 1-MMP-dependent tumor growth in mice, *Cancer Res*, **67** (2007), 4311–4319.
[12] A. Watanabe, D. Hosino, N. Koshikawa, M. Seiki and T. Suzuki, et al., Critical role of transient activity of MT1-MMP for ECM degradation in invadopodia, *PLoS Comput Biol*, **9** (2013), e1003086.
[13] A. M. Weaver, Invadopodia: Specialized cell structures for cancer invasion, *ClinExpMetastasis*, **23** (2006), 97–105.

Received October 15, 2014; Accepted June 19, 2015.

E-mail address: kichi@ims.u-tokyo.ac.jp