A New Approach for Estimating the Progression of Pancreatic Cancer

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Abstract—Cancer of the pancreas is a highly lethal disease and has an extremely poor prognosis. It is the fourth leading cause of death from cancer in the US and the twelfth worldwide. There are currently only few therapeutic options for patients with pancreatic cancer. Hence new insights into the pathogenesis of this lethal disease are urgently needed. In recent years, extensive biological research has been conducted to study the mechanisms that control the initiation and progression of pancreas cancer. Mathematical models have also been used to present quantitative analysis and predict reasonable time schemes for the progression of pancreatic cancer. However, in those published articles, it was assumed that the mutation rate was constant, which is not realistic. In this work, we present a new approach using non-constant mutation rate and hence reveal several important biological parameters of cancer progression, such as initial mutation rate as well as doubling time (or selective advantage coefficients) in different stages, and eventually present a better time scheme. Under more realistic assumptions regarding gene mutation and a more reasonable mutation rate, the averaged values of doubling time and selective advantage coefficient generated by our model are consistent with the predictions made by the published models.

I. INTRODUCTION

Pancreatic cancer remains a major challenge for all of us. It is the fourth leading cause of death from cancer in the US and the twelfth worldwide, with an estimated 37,680 people diagnosed with the disease and 34,280 people dying from the disease each year [1], [7]. Cancer of the pancreas is a highly lethal disease, with ductal adenocarcinoma being the most common histologic type, and has an extremely poor prognosis. It has the worst 1 and 5 year survival of any cancer: for all stages combined. The overall 5-year survival has not been improved in the past 30 years but remains ≤ 6%. Median survival is approximately 6 months for patients with metastatic disease and 10 months for patients with locally advanced disease. Of approximately 50% of patients with pancreatic adenocarcinoma who present with clinically apparent metastatic disease, only a minority (10%−20%) of patients are considered resectable [2], [9]. 50% of patients die of recurrent tumor within 2 years. In addition to a poor survival rate, patients with pancreatic cancer have a great deal of suffering, with a particularly high incidence of pain, mostly caused by a predilection for the tumor to invade the perineural space of nerves in the celiac plexus [18]. In addition, substantial weight loss and multiple gastrointestinal symptoms sap the energy of patients with the disease. Even after curative radical surgery,

the recurrence rate is very high: 5-year survival rates was only 10% − 20% and liver metastasis occurred within 6 months in 60.9% of patients and within 1 year in 95.1% of patients [8], [11], [12]. In a comprehensive genetic analysis of 24 patients' pancreatic cancers, Jones et al. noted an average of 63 genetic alterations in each tumor, the majority of which were point mutations [5]. However, it was known that factors for predicting long-term survival following resection include clear surgical margins, small tumor size (≤ 2 cm), negative lymph nodes, and reduced perioperative morbidity [2]. There are currently few therapeutic options for patients with pancreatic cancer. Therefore new insights into the pathogenesis of this lethal disease are urgently needed.

Cancer progression is a complex series of steps including mutations, mutated cell cloning, further mutations, reaching the maximal tumor size and then leaving the original tumor site. In additional, some cancer cells have the ability to metastasize and migrate to other parts of the body via the bloodstream, the lymphatic system, or by direct extension. Finally the migrated cancer cells land in a new location and develop into a new tumor (metastasis).

Based on these observations, Jones et al. [6] investigated the common mutational patterns during the phases of tumor initiation, invasion and metastasis, to evaluate the time extending through each phase. To present a reasonable time scheme for cancer progression, they considered three critical time points: (i) the one (FcellMet) that gave rise to the final clonal expansion resulting in the index metastasis; (ii) the last common ancestor (FcellACa) of the advanced carcinoma and FcellMet; and (iii) the last common ancestor of the large adenoma and FcellACa of the cancer progression. Then they calculated the time required for each interval: the interval between the birth date of a founder cell for a large adenoma and that of the founder cell of an advanced carcinoma, and the interval between the birth date of a founder cell of an advanced carcinoma and that of the founder cell of the index metastasis. For example, the time required for the interval between the birth date of a founder cell for a large adenoma and that of the founder cell of an advanced carcinoma is given by

$$\Delta T_{LAd,ACa} = F_{Lad,Aca} \cdot T_{ACa}, \qquad (1)$$

where $F_{Lad,Aca}$ is the fraction of mutations in the advanced carcinoma that were not found in the large adenoma, and T_{ACa} is the birth date of the founder cell of advanced carcinoma.

For example, patient Mx34 was 83 years old when she developed an advanced carcinoma of the ascending colon that was 9 cm in diameter and of stage T4N2M1 (here N2 indicates that cancers cells were found in more than three mesenteric lymph nodes) [6]. A residual adenoma that surrounded the carcinoma was identified at the time of surgery. A small (1 cm diameter) mesenteric lymph node metastasis was found to contain 25 mutations that were subsequently evaluated in other lesions of this patient. Of these, 24 were found in the colorectal carcinoma ($F_{ACa,Met}$= 0.04). The evaluation of the same mutations in the large adenoma from which the carcinoma developed revealed an $F_{LAd,ACa}$ of 0.23. Application of Equation (1) indicated that the large adenoma founder cell was born 17 years before the advanced carcinoma founder cell. In the 17 years between the birth of $F_{cellLad}$ and $F_{cellACa}$, the tumor underwent waves of clonal expansion driven by mutations in TP53 and the other genes presumably required for invasion and further growth of this tumor. Once it acquired these capabilities, a cell ($F_{cellMet}$) capable of lymph node metastasis appeared within a relatively short period. Jones et al suggested that the following general conclusions about colorectal tumorigenesis: namely, it takes around 17 years for a large benign tumor to evolve into an advanced cancer but < 2 years for cells within that cancer to acquire the ability to metastasize [6].

Yachida et al presented a more reasonable theory for the stages of pancreatic cancer progression and also a quantitative analysis of the timing of the genetic evolution of pancreatic cancer [17]. The result indicates at least a decade between the occurrence of the initiating mutation and the birth of the parental, non-metastatic founder cell. At least five more years are required for the acquisition of metastatic ability and patients die an average of two years thereafter. The results showed that the pattern observed in the cells that originated metastasis were clearly represented in the cells within the primary carcinoma. In addition, using the following mathematical model:

$$T = \frac{T_{gen}}{r}(N_1 + \sqrt{N_1})$$

where $T_{gen} = 2.3\,\text{days}$ and $r = 0.016$ per generation, Yachida et al. calculated the elapsed time between the different stages of the tumorigenic process. The result is very similar to that reported by Jones et al [6], with an average of 11.7 years from the initiation of tumorigenesis until the birth of the cell giving rise to the parental clone, an average of 6.8 years from then until the birth of the cell giving rise to the index lesion, and an average of 2.7 years from then until the patients' death. Taking these correlations as a conservative assumption, the knowledge of the dynamics of the tumor progression, in quantitative terms, offers an opportunity to interfere in the tumor evolution and develop a more customized treatment.

Yachida et al calculated the timing of pancreatic cancer in [17]. Here we present their results in Table 1. In this table, T_1 is the time between tumor initiation and the birth of the cell giving rise to the parental clone, T_2 the subsequent time

required for the birth of the cell that gave rise to the index metastasis, and T_3 the time between the dissemination of this cell and the patients' death. In addition, we add the survival time from the initial diagnosis of each patient, which is the last column of Table 1.

This time table is reasonable except the result for patient Pa03c, because we might have the inequality $T_1 \geq T_2 \geq T_3$ from the definition. This is probably due to the assumption of the constancy of mutation rate made in [17]. We will present a similar result by using a different approach of non-constancy mutation rate.

II. METHODS

Tumorigenesis can be regarded as an evolutionary process, in which the transformation of a normal cell into a tumor cell involves a number of limiting genetic and epigenetic events. To study the progression process, a time scheme has been presented for colorectal cancer by an extensive clinical investigation. Moreover, mathematical models have been designed to describe this biological process. However, these models assumed that mutation rate is constant during different stages. In fact it has been pointed out that the subsequent driver mutations appear faster than the previous one and the cumulative time to have more driver mutations grows with the growing mutation number. Thus it is still a challenge to calculate the time when the first mutation occurs and to determine the influence of tumor size on the mutation rate. In this work we present a general framework to remedy the shortcoming of existing models.

According to the multistage theory, cancer is the last stage of a series of k sudden and irreversible changes which must take place in a cell in a specific order. Denote i-cell as a cell with i mutations, $p_i(t)$ the fraction of all i-cells in the whole population, $\mu(t)$ the mutation rate at time t and t_i (let $t_0 = 0$) the time point for the first i-mutated cell (a cell having exact i- mutations) appears. Although the time points t_i are random in essence in the cancer stochastic progression, at this stage we will follow the deterministic approximation approach. This approach was proposed and discussed in details by Beerenwinkle et al (Supporting Information Material "Analysis approximations for the expected waiting time" to [3]), by which not only over-complicate details can be avoided, the information would not be lost too much if the population is not too small. Thus, $\{t_i\}$ are assumed to be deterministic and during the small interval $[t_{i-1} - t_i]$ the value of function $\mu(t)$ is

$$\mu(t) \approx \mu(t_i) \equiv \mu_i. \qquad (2)$$

We neglect the probability of two or more events taking place in (t , t+dt) as $dt \to 0$. If a cell is in state p_i at time t_i, the probability of transformation to state p_{i+1} in a small time interval Δt is given by

$$\mu(t_{i+1})\Delta t + o(\Delta t),$$

$o(\Delta t)/\Delta t \to 0$ as $\Delta t \to 0$. Also, the probability of transformation from state i to state $i + j$ with $j > 1$ in time Δt is

TABLE I

ESTIMATES OF TIME IN THE CLONAL EVOLUTION OF METASTATIC PANCREAS CANCER [17]. UNLESS INDICATED, THE UNIT OF TIME IS YEAR.

Patient	T_1	T_2	T_3	Total tumor time	survival from diagnosis
Pa01c	16.1(2.5)	9.8(2.0)	2.9(1.2)	28.8(3.4)	6 month
Pa02c	10.6(2.0)	9.4(1.9)	2.7(1.2)	22.7(3.0)	8 month
Pa03c	7.9(1.8)	2.4(1.0)	2.7(1.2)	13.0(2.4)	1 month
Pa04c	11.4(2.1)	7.9(1.8)	2.7(1.2)	22.0(3.0)	7 month
Pa05c	9.1(1.9)	4.3(1.3)	2.3(1.2)	15.7(2.6)	10 month
Pa07c	15.7(2.5)	3.1(1.1)	2.7(1.2)	21.5(3.0)	3 month
Pa08c	11.4(2.1)	10.6(2.0)	2.7(1.2)	24.7(3.1)	15 month
Average	11.4(2.1)	6.8(3.4)	2.7(1.2)	21.2(4.8)	7.1 month

assumed to be $o(\Delta t)$. This implies that $1/\mu_{i+1}$ is the averaged time required for a cell to go from state i to state $i+1$. Thus the probability to find a cell in the ith stage by the end of time interval $(t, t+dt)$ is given by

$$p_i(t+dt) = (1 - \mu_{i+1}dt)p_i(t) + p_{i-1}(t)\mu_i dt.$$

Taking the limit $dt \to 0$, the above equation becomes

$$\frac{dp_i(t)}{dt} = \mu_i p_{i-1}(t) - \mu_{i+1}p_i(t), \quad i = 2, \cdots, t_K. \quad (3)$$

and for the case $i = 1$, we have

$$\frac{dp_1(t)}{dt} = -\mu_1 p_1(t).$$

The system is complete with given initial conditions $p_1(0) = 1$ and $p_i(0) = 0$, $i = 2, \cdots$, which mean that all cells were normal at time $t = 0$.

We now assume that the mutation rate $\mu(t)$ have the form of

$$\mu(t) = u_0 e^{ast}, \quad (4)$$

where s is the selective advantage coefficient, u_0 the initial mutation rate, and a the transform factor linking the selective advantage coefficient and the mutation rate [14]. Note that both s and a vary with individuals and the product $b = as$ is determined by the curvature of $\mu(t)$. Thus we have that

$$\mu_j \leq \mu_{j+1}.$$

Since $\mu_j - \mu_{j+1} = \mu(t_j) - \mu(t_{j+1})$ usually is small, we have the following

$$\begin{aligned} \mu_j p_{j-1} - \mu_{j+1}p_j &= (\mu_j - \mu_{j+1})p_j + \mu_j(p_{j-1} - p_j) \\ &\approx \mu_j(p_{j-1} - p_j) \\ &\approx \mu(t)(p_{j-1} - p_j) \end{aligned}$$

for $t_{j-1} \leq t \leq t_j$. Thus we have the approximation

$$\frac{dp_j}{dt} = \mu(t)(p_{j-1} - p_j), \quad j = 1, 2, \cdots, t_K$$

for $t_j \leq t \leq t_{j+i}$. Then the solution of the above system is

$$p_j(t) = \frac{\lambda(t)^j e^{-\lambda(t)}}{j!} \quad (5)$$

where

$$\lambda(t) = \int_0^t \mu(x)dx = \frac{\mu_0}{as}(e^{ast} - 1).$$

Finally, the time point t_k, at which the first k-mutated cell (a cell having exact k mutations) appears, is

$$t_k = \frac{\ln(\frac{as\lambda_k}{\mu_0} + 1)}{as}. \quad (6)$$

where $\lambda_k = \lambda(t_k)$.

For time point t_k (at which the first k-mutated cell appears), we have $p_k = p_k(t_k) = \frac{1}{N}$, where N is the total number of sensitive cells which also is the population size we are discussing here. Using Equation (5), we have

$$\frac{1}{N} = \frac{\lambda_k^k e^{-\lambda_k}}{k!}$$

and hence

$$\lambda_k = -k \cdot \mathrm{LambertW}(-\frac{k!^{1/k}}{kN^{1/k}}) \quad (7)$$

where LambertW is the principal branch of the Lambert W function, which is the inverse function of the function $f(x) = xe^x$ where e^x is the exponential function and x is a complex number.

Note that when $s \to 0$, t_j approaches to $\frac{\lambda_j}{\mu_0} = \frac{-k}{\mu_0} \cdot \mathrm{LambertW}(-\frac{k!^{1/k}}{kN^{1/k}})$ which is consistent with a result of Beerenwinkle et al [3], but very obviously our structure significantly extends their's.

III. RESULTS

Using our new method, we calculate the data of seven pancreas patients and present a time table which is very similar to that in [17]. However, we do not need the value of 0.016 for the average mutation rate which was crucial in [17].

Assume that each individual cell doubles in every cell division. If the tumor diameter is r cm, the selective advantage coefficient s satisfies the following condition

$$s = 2^{(3\log_2 r + 9\log_2 10)/t} - 1. \quad (8)$$

If not all cells divide in each cell cycle, the value of s becomes smaller. So expression (13) is an upper bound.

For the T_1-stage, the average doubling time $DT(T_1)$ of the tumor follows the following formula

$$DT(T_1) = \frac{\ln 2}{\ln(s+1)} \qquad (9)$$

Thus for patient Pa01c, the average doubling time of the T_1-stage is

$$\frac{\ln 2}{\ln(1.0134)} = 150.$$

Similarly, we can calculate the doubling time of other patients. The results are presented in Table 2.

For the T_2-stage, the average doubling time $DT(T_2)$ of the primary tumor follows the following formula

$$DT(T2) = \frac{(t2*356 - 20*2.3)}{(\text{number of doubling} - 20)2} \qquad (10)$$

where 2.3 is the average cell-doubling time (or cell division time). The reason to include a factor $\frac{1}{2}$ in equation (15) is that the total time t_2 of the T_2-stage is roughly the double time of the formulation of the primary tumor by the definition of the T_2 stage.

Thus for patient Pa01c, the average doubling time of the primary tumor is

$$\frac{(9.5*356 - 20*2.3)}{17.6*2} = 95 (\text{days}).$$

Similarly, we can calculate the doubling time of the primary tumor for other patients and the results are presented in Table 2. Numerical results in Table 2 suggest that the average doubling time of the T_1-stage is longer that of the T_2-stage, which is consistent with the clinical observation.

The sensitive cells here mean the cancer stem cells that like normal stem cells having the ability for self-renew. However, cancer stem cells have lost many of the cell division control mechanisms under which normal stem cells operate. Because cancer stem cells do not control their cell division properly, they may give rise to tumors. In addition, the progeny of cancer stem cells do not differentiate properly. The progeny tend to be relatively immature or unspecialized, and thus do not function as well as they should in carrying out normal body functions.

The estimation of the size of metastatic tumor is based on the number of doubling of the metastatic tumor using the formula:

$$\frac{(t3*356 - 20*2.3)}{56} + 20. \qquad (11)$$

where 56 (generations) is the median doubling time of pancreatic cancer metastases reported by Amikura et al [2] in which a two stage model was used. We estimated that the tumor doubling time equals to the cell doubling time until the tumor size reached 1 millimeter in diameter at which time angiogenesis is required [15]. Thereafter, we used the average doubling time described above and the following formula to calculate the tumour size

$$\text{diameter of tumor} = \frac{1}{100}e^{\frac{\ln 2*\text{number of doublings}}{3}} (\text{cm}). \qquad (12)$$

For example, for patient Pa01c, the number of doubling is about

$$\frac{(2.9*356 - 46)}{56} + 20 = 37.6$$

Then using formula (17), we find that the upper bound of the diameter of the tumor is

$$\frac{1}{100}e^{\ln 2*37.6/3} = 5.9 \,(\text{cm}).$$

Similarly we can calculate the tumor size for other patients. The results are presented in Table 3.

To calculate the selective advantage coefficient s_3 in the T_3-stage, we use the formula

$$s_3 = 2^{\frac{1}{DT}} - 1.$$

For patient Pa01c, we have

$$s_3 = 2^{1/37.6} - 1 = 0.06.$$

Similarly we can calculate the selective advantage of metastasis for other patients. The results are presented in Table 3.

Our calculation supports the view that tumor size is an important predictor for prognosis. For example, patient Pa05c had the smallest tumor size (2.5 in diameter). The patient also had second longest survival time – 10 months after diagnosis. However, other six patients had 6 months, 8 months, one month, 7 months, 3 months and 15 months, respectively (see Table 1).

IV. CONCLUSION AND DISCUSSION

Since cancer of the pancreas is a highly lethal disease, new insights into the pathogenesis of this lethal disease are urgently needed. In this work we proposed a new approach to study the timing of pancreatic cancer, which presents useful information of parameter values for medical treatment. The feather of our approach is that the mutation rate is a function of time, rather than a constant that has been used in literature [6], [17]. Using this approach, we calculated the initial mutation rate, doubling time or selective advantage coefficients in different stages of cancer. Compared with existing results, our results presents a better approximation under more realistic assumptions. The analysis in this work suggested our approach is a promising method to study the timing of cancer and hence has a potential application to medical treatment.

Previously, Meza et al. [10] reported the average sojourn time of premalignant pancreatic lesions to be 50 - 60 years based on the analysis of pancreatic cancer incidence data, which is significantly longer than we calculated. Yachida et al [17] reported the average sojourn time of premalignant pancreatic lesions to be 21 years which is more reasonable and is close to our calculation. The significant difference between the times in [17] and [10] may be due to the fact that the times presented in [17] were based on data from patients that developed detectable pancreatic cancers. However, Meza et al. reported the average sojourn times for all premalignant pancreatic lesions, including those that never become malignant during an individual's lifetime. Our calculation supports the conclusion made in [17]. Since our approach does not assume

TABLE II

ESTIMATES OF TIME IN THE CLONAL EVOLUTION OF METASTATIC PANCREAS CANCER. DT(T_2) STANDS FOR THE AVERAGE DOUBLING TIME OF THE PRIMARY TUMOR.

Patients	T_1	T_2	b	μ_0	DT(T_1)	DT(T_2)	sensitive	s_1	$\overline{\mu}$
Pa01c	14.6	9.5	0.0008	0.0052	150	95	10^7	0.0134	0.0117
Pa02c	10.9	8.4	0.0007	0.0054	140	90	10^6	0.014	0.0103
Pa03c	10.5	7.8	0.0012	0.0086	113	84	10^5	0.018	0.009
Pa04c	10.8	8.5	0.0006	0.01	137	91	10^6	0.0172	0.01
Pa05c	10.5	8.2	0.0012	0.0086	113	104	10^5	0.018	0.0086
Pa07c	13.6	8.6	0.001	0.013	148	92	10^7	0.0136	0.0129
Pa08c	12.5	9.8	0.001	0.009	135	105	10^6	0.0149	0.009
Average	11.0	8.7	2.7	23.3		94.3	10^6	0.0151	0.0102

TABLE III

ESTIMATES OF TIME IN THE CLONAL EVOLUTION OF METASTATIC PANCREAS CANCER. DT(T_3) STANDS FOR THE AVERAGE DOUBLING TIME OF THE METASTASIS TUMOR. s_3 STANDS FOR THE AVERAGE SELECTIVE ADVANTAGE OF METASTASIS.

Patients	T_1	T_2	T_3	total-time	tumor-size	DT(T_3)	s_3
Pa01c	14.6	9.5	2.9	27	5-6 cm	37.6	0.06
Pa02c	10.9	8.4	2.7	22	4-4.5 cm	36.4	0.062
Pa03c	10.5	7.8	2.7	21	4-4.5 cm	36.4	0.062
Pa04c	10.8	8.5	2.7	22	4-4.5 cm	36.4	0.062
Pa05c	10.5	8.2	2.3	21	2.5 cm	33.8	0.068
Pa07c	13.6	8.6	2.7	25	4-4.5 cm	36.4	0.062
Pa08c	12.5	9.8	2.7	25	4-4.5 cm	36.4	0.062
Average	11.0	8.7	2.7	23.3		36	0.063

the constancy of the mutation rate, our study could reveal more biological insights than the published model in [17]. Besides the sojourn time, we are able to calculate other important biological parameter, such as initial mutation rate and doubling time (or selective advantage coefficients) in different stages. Our results suggested that our model is more realistic and reasonable presenting a better approximation.

Therefore our model may provide answers to several questions about pancreatic tumorigenesis that have long perplexed researchers and clinicians. For example, why is there so much heterogeneity in the times required for tumor progression among different patients? Why is there so much heterogeneity in the sizes and development times of tumors even within individual patients? Why do the majority of patients with pancreatic adenocarcinoma present at an advanced stage at the time of diagnosis?

Our model is compatible with the view that a few major mutational pathways, such as those involving KRAS, TP53, CDKN2A and SMAD4 [3][16], endow relatively large increases in fitness that can allow tumors to grow to sizes compatible with further progression. However, the final course to malignancy will be determined by multiple mutations, each with a small and distinct fitness advantage, and these mutations occur stochastically. Every cancer will thereby be dependent on a unique complement of mutations that will determine its propensity to invade (its ability to metastasize). Our approach also suggested that the biological heterogeneity of cancer may be a direct consequence of the tumorigenic process itself [15].

Amikura [2] suggested that the early development of liver metastases within 3 month after pancreatic resection supports the hypothesis that occult microscopic liver metastases are frequently present at the time of resection. A recent study [13] reported that undifferentiated pancreatic ductal adenocarcinoma is independently associated with hepatic metastasis after pancreatic resection. This conclusion is also consistent with the one that tumor size was the most important factor for liver metastasis made in [8]. Based on our calculation of those biological parameters, it is possible for us to present an optimal medical treatment scheme. According to tumor size, we may process resection to prevent tumor metastasis. More research work is needed to determine the detailed time of the treatment, which will be the topic of our future work.

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