Systems Medicine Lecture Notes

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Lecture 7

Aging-related diseases and their exponentially rising incidence with age

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Many diseases occur at young ages, including autoimmune diseases we discussed in part 1, such as T1D which has a maximum incidence around age 14. In addition, there is a large number of diseases whose incidence rises with age, called **age-related diseases**. Examples include cancer, failure of specific organs such as cardiac failure, kidney failure and lung failure, neuro-degenerative diseases such as Alzheimer and Parkinson, osteoarthritis and type 2 diabetes. With ageing also comes weakened muscle strength, susceptibility to infection, and slow healing from injury. Age-related diseases are the major causes of death in the past 100 years, as the population ages.

Age-related diseases show a common pattern in their incidence rate. **Incidence** is the probability to get the disease at a given age among all people who survive to that age. The incidence of these diseases rises exponentially with age, and drops at very old ages (Fig 7.1). The slope of this exponential increase is similar for different disease, around 6-7% per year. Understanding this exponential rise is a major aim of this lecture. What is different about the decade of age 20-30 and the decade of age 70-80 that makes these diseases so much more likely?

The age-related disease are major killers, as seen by the risk of death plotted by different causes (Fig 7.2). The risk of dying from each of the diseases also rises exponentially with age.

We will start with a well-studied age-related disease in which lungs fail, called IPF. Its fundamental cause is a mystery. The

Figure 7.1

Figure 7.2

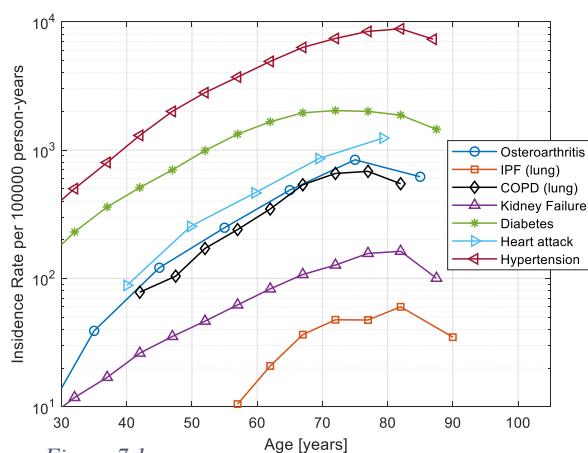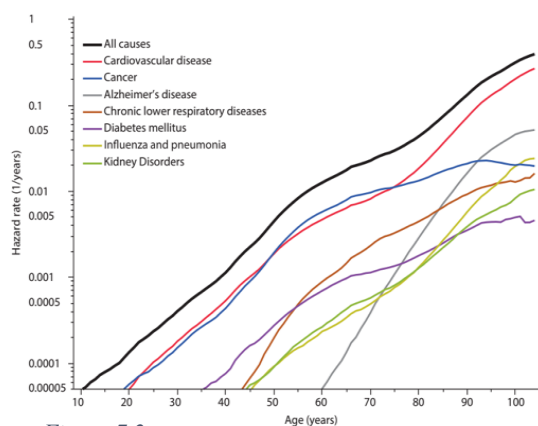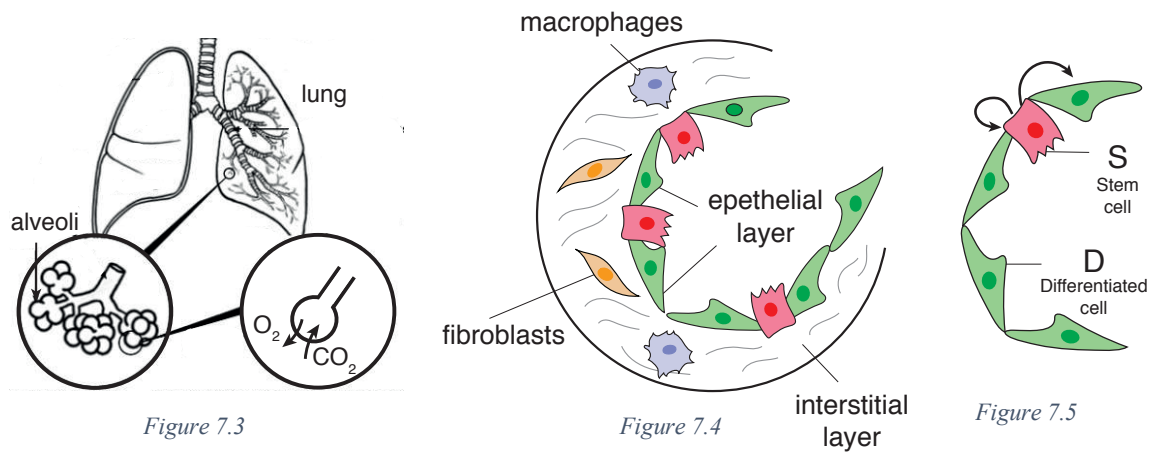prevalence of IPF prevalence is about 10^{-4}, and its incidence rises exponentially with age,

and drops after age 80 (Fig 7.1). We will attempt to explain this disease as an outcome of fundamental principles of tissue homeostasis, using the concepts we learned in the past two lectures. We will then discuss other age-related diseases.

Theory for IPF: IPF stands for **idiopathic pulmonary fibrosis**. Breaking this down, 'idiopathic' means disease of unknown cause, 'pulmonary' means of the lungs, and 'fibrosis' means excess scarring. In IPF, lung capacity is progressively lost due to the scarring of tissue that is essential for breathing.

To understand IPF, lets survey the relevant tissue structure. The lung is made of branching tubes that end in small air sacks called **alveoli** (Fig 7.3) The alveoli function to move oxygen from the air to the blood, and to let CO_2 from the blood out into the air. The alveoli are made of two layers- an inner epithelial layer that is one-cell thick, and an interstitial layer. IPF scarring occurs in the interstitial layer surrounding the alveoli (Fig 7.4).

The epithelial layer is made of two types of lung cells called alveolar type 1 and 2 (AT1, AT2) (Fig 7.5). We will call the first type, which are large flat barrier cells, the differentiated cells D. The second type are smaller stem cells we will call S. These stem cells can divide to form new S cells, or to form new D cells. The S cells also secrete a soapy surfactant that shields the cells from the air, protects the cells from air particles and also prevent collapse of the alveoli when we exhale.

Figure 7.3

Figure 7.4

Figure 7.5

The interstitial layer around the alveoli is made of elastic fibers that provide mechanical strength to the alveoli. This layer contains two other cell types, fibroblasts and macrophages. These two cell types will be the stars of the next lecture. Macrophages ('big eaters') are ready to gobble up bacteria and particle if they make it through the epithelial layer of S and D cells. The **fibroblasts** produce the fibers which make the elastic sheath around the alveoli.

When there is injury to the D cells, they signal (using molecules such as TGF-beta) to S cells to differentiate into new D cells (Fig 7.6). The injury signal also causes S cells to activate a repair process in the interstitial layer around
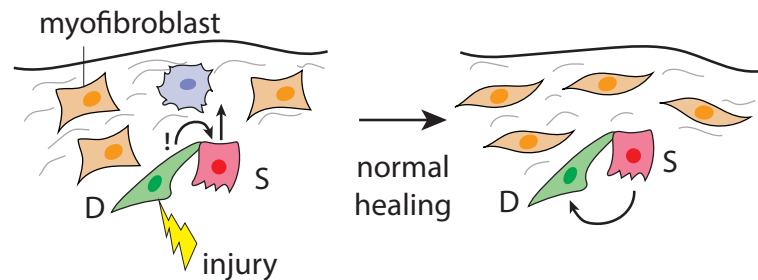

Figure 7.6

the alveoli. The S cells signal the fibroblasts to become activated, proliferate and secrete extra fibers. The activated fibroblasts are called **myofibroblasts**. In normal healing, once the new D cells are made, the excess fibroblasts commit programmed cell death and the extra fibers are removed. The injury is healed.

In IPF, an unknown factor causes an ongoing injury. The S cells multiply and reach higher numbers than in normal alveoli (Fig 7.7). They activate the fibroblasts to multiply and lay down excessive fibers. These excess fibers form a scar, known as **fibrosis**. Thus, IPF causes the interstitial tissue around the alveoli to become a thick fibrotic scar that reduces the ability of oxygen to flow from the lung to the blood, and the ability of CO_2 to be ventilated out. It makes the alveoli stiff and less able to expand and contract. Eventually more and more alveoli become dysfunctional. Lung capacity is

Figure 7.7

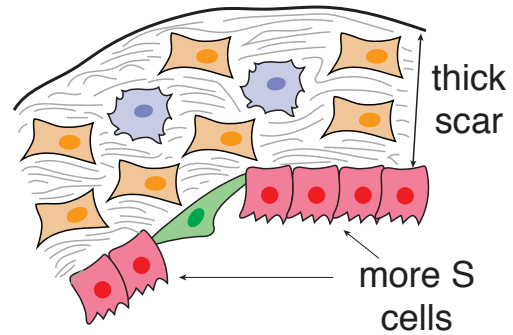progressively lost, and patient dies within 1- 3 years. IPF is a chronic progressive disease that has no cure.

The unknown in IPF is the origin of the injury. We can use what we have learned so far to make a theory to address the source of the injury, and explain why the risk of IPF rises exponentially with age, and why it occurs in only a small fraction of the population. We rely on recent work (2017,2019) that shows that senescent cells (SnC) are important for IPF: Removing SnC improves IPF in a mouse model (IPF induced by Bleomycin). We will thus explore how SnC can cause IPF. The main idea is that SnC slow down the rate of stem cell proliferation; when proliferation drops too low, the alveolar tissue first enriches with S cells, and then locally crashes.

In labile tissues, stem cells must self-renew and also supply differentiated cells

To understand IPF, we thus need to understand how stem cell-based tissues work. Such stem-cell tissues are different from the tissues we discussed in the first part of the course. There, we considered **stable** tissues in which a cell type divides to make more of itself. For example, beta cells give rise to beta cells (Fig 7.8).
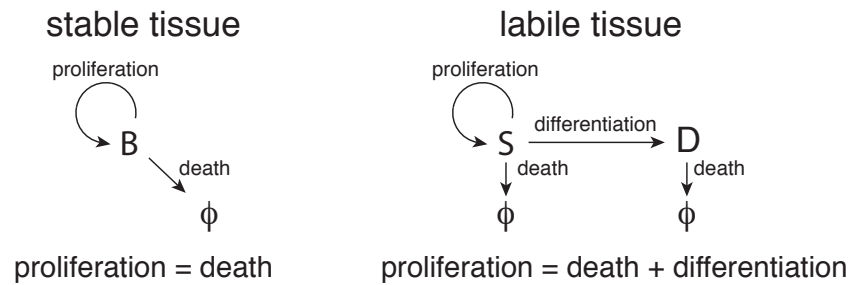

Figure 7.8

The lung is an example of a tissue with stem cells and differentiated cells, called a **labile tissue.** Labile tissues are often found in organs exposed to the outside world like the lung, intestine and skin. Because of this exposure, cells can be damaged and need to be replaced. These tissues divide labor: the majority of cells D do the main tissue work, and the minority (1-5%) are stem cells S who carry out most of the divisions to regenerate the D cells. Thus

$S \rightarrow D$. In some labile tissues, such as blood and skin, there is a series of differentiated cells $S \rightarrow D_1 \rightarrow D_2 \rightarrow \ldots \rightarrow D_n$. Some of these intermediate cell types can undergo a limited number of divisions, and are called 'transient amplifying cells'. This elaborate structure does not seem to exist in the alveoli.

Recall that in stable tissues such as beta cells, steady-state requires that cell proliferation rate equals cell death rate, otherwise the tissues grows or shrinks. In contrast, in labile
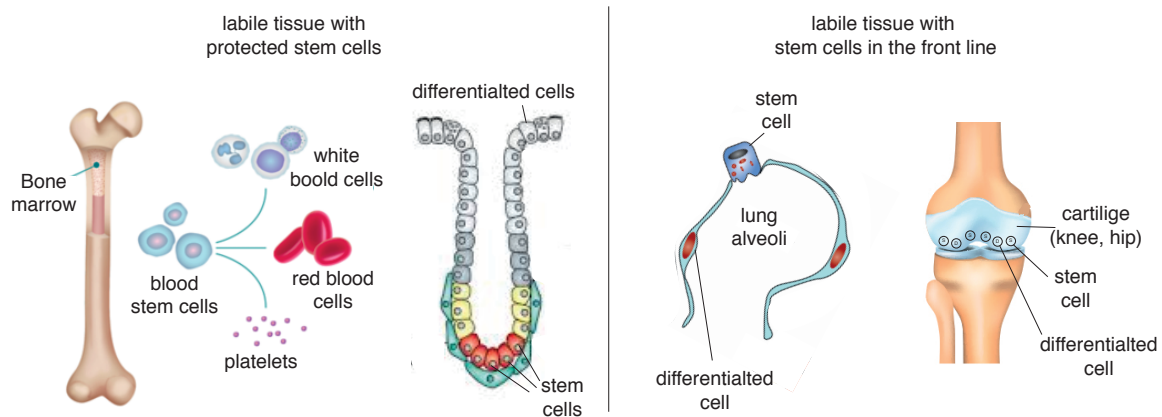

Figure 7.9

tissues S proliferation must *exceed* S death, because some of the S divisions are needed to make the D cells. For stem cells, therefore, proliferation must balance two processes: death plus differentiation.

Stem cell death rate, in many labile tissues, is relatively low because the stem cells are in a protected niche, where they are shielded from damage. Examples include the blood stem cells hidden in the bone marrow, skin stem cells in the deep epithelium, and the gut stem cells tucked away at the bottom of crypts (Fig 7.9).

In contrast, the lung alveoli is an example of a labile tissue where both S and D are on the front lines. Both are exposed to damage, such as air particles, pathogens and the mechanical stress of breathing. There is no other choice: the alveoli must be thin to allow diffusion of gases, and can't afford a deep layer for the stem cells.

As an aside, we can dream of a 'Mendeleev table' of diseases according to cell type circuit design, such as front line versus protected niche labile tissue, versus stable tissues. Each type of tissue might be prone to different types of diseases. There might be missing diseases, and predicted diseases.

We are now ready to write down the basic equations for a labile tissue. These equations account for stem-cell S proliferation at rate p, and their differentiation to make differentiated cells D at rate q. The death rates of S cells is d_1, and of D cells is d_2:

$$(1) \quad \frac{dS}{dt} = pS - d_1 S - qS$$

$$(2) \quad \frac{dD}{dt} = qS - d_2 D$$

Note that differentiation means a loss of an S cell and a gain of a D cell. As a result, the $-qS$ term in the first equation shows up as a $+qS$ term in the second.

There are additional subtleties of whether each stem-cell division is symmetric (yielding two S or two D cells) or asymmetric (yielding an S and a D cell). These subtleties do not matter for the present discussion (exercise 7.5).

In order to keep the tissue at homeostasis, and in particular to maintain a proper concentration of D cells, labile tissues have an additional feedback loop. In this feedback loop, the cells D and S signal to each other by secreting molecules that affect differentiation and proliferation rates. If there are too few D cells, for example, these signals act to increase D cell production and restore homeostasis.

In a typical feedback loop found in the lung and skin, D secretes a signaling molecule that increases S differentiation (one such molecule is $TGF\beta$, a strong signal for differentiation). Similarly, S secretes factors that increase its own differentiation rate. Thus, differentiation rate is an increasing function of D and S concentrations, $q = q(S, D)$.

Let's see how this feedback works. Suppose there is a loss of D cells (Fig 7.10). Since D cells signal to increase differentiation, less D cells mean lower differentiation r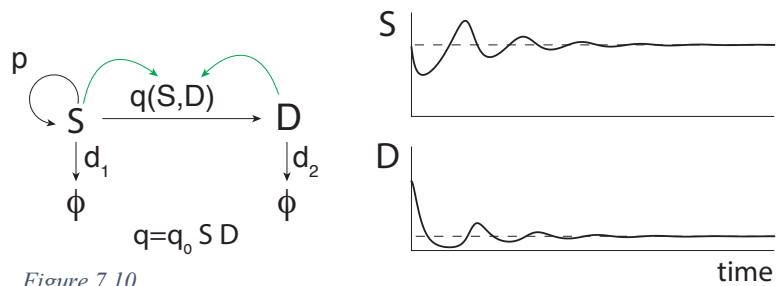ate q. Thus, there are even fewer D cells. But the reduction in differentiation means that more S divisions go to making new S cells instead of D cells. S levels rise, and eventually the larger S cell population supplies more differentiation events per unit time than before. D levels rise back. The timescale is months, due to the turnover rate of about a month of alveolar epithelial cells.

Figure 7.10

This feedback process shows damped oscillations and settles down to a proper steady state. As an aside, we can predict, as in chapter 4, that such damped oscillations might entrain to the seasons and lead to seasonal changes in alveolar composition, with more S cells and thus more surfactant in some seasons and less in others.
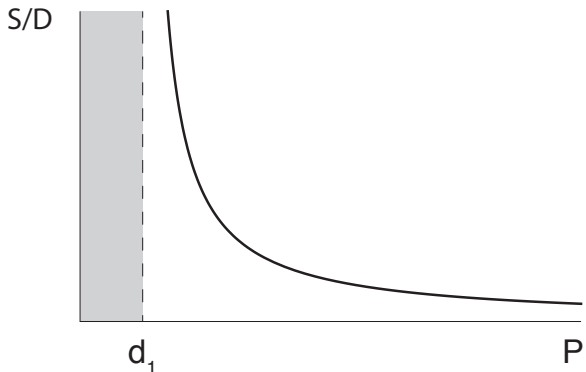

In Fig 7.10, by the way, we used a concrete example of such as feedback loop, in which $q(S, D) = q_0 S D$.

Figure 7.11

when proliferation drops to approach stem cell removal d_1. Thus, the higher the S death-rate d_1, the number of S cells rises to compensate. The ratio of stem to differentiated cells, $\frac{S_{st}}{D_{st}} = d_2/(p - d_1)$, rises with cell death rate d (7.11). In typical healthy alveoli tissue (16%

AT2 cells, 8% AT1 cells but AT2 cells 7% of surface area (*Am Rev Respir Dis*. 1982), $S/D = 2 = 1/(p/d - 1)$ thus p/d~1.5. The rise in S cells buffers the decrease in D cells due to the rise in death d (Fig 7.12).
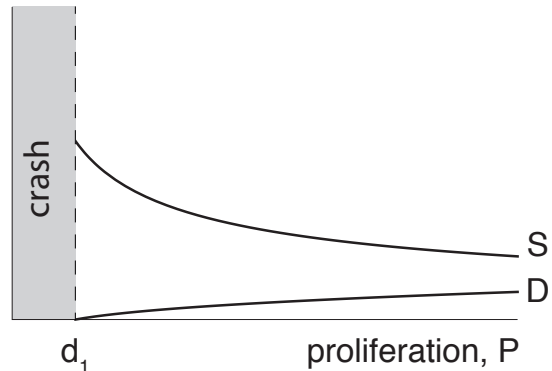
The basic equation structure for labile tissue with sizable stem cell death has a fragility. When proliferation rate of stem cells drops below their death rate, $p < d_1$, a catastrophe happens- the tissue collapses. To see this, we can sum the two equations (1) and (2) to get an equation for total cell number S+D:

Figure 7.12

$$\frac{d(S + D)}{dt} = p\,S - d_1\,S - d_2\,D$$

We assume that the feedback provides a steady state solution. Finding this solution, $d(S + D)/dt = 0$, we find that, no matter what the feedback loop is, the ratio of S to D cells rises and diverges as proliferation p approach stem cell death d1:

$$\frac{Sst}{Dst} = \frac{d_2}{p - d_1}$$

Thus, the fraction of S cells in the tissue diverges as proliferation drops towards the S-removal rate d1 (Fig 7.12). There are more and more stem cells relative to D cells, to supply the needed amount of D cells, as well as their own renewal. Elegantly, the rising amounts of S cells also lead to more surfactant that protects the alveolus. In IPF, this is a doomed attempt to prevent tissue collapse.

The ability of S cells to produce the needed amount of cells for the tissue breaks down completely when proliferation p falls below the death rate d1. The cell population crashes to zero.

To see this mathematically, we take our equation, and bound it by a simpler equation which crashes. We first increase the right-hand-side by using the smaller of the two death rates (let's say without loss of generality d1<d2)

$$\frac{d(S + D)}{dt} = pS - d_1\,S - d_2\,D \;<\; pS - d_1(S + D)$$

We can further increase the rhs by changing S to S+D because D is always positive

$$\frac{d(S + D)}{dt} < p(S + D) - d(S + D) = (p - d_1)(S + D)$$

We end up with a simple linear equation for total number of cells T=S+D which goes as

$$\frac{dT}{dt} = (p - d_1)T.$$

Thus, when $p < d_1$, the total cell number is bounded below an equation that goes to zero exponentially fast with time. This makes sense: in each unit time, addition of new cells goes as S renewal, and removal of cell mass goes as death. Renewal rate must not go below death rate or there is exponentially fast loss of cells.

Senescent cells slow down proliferation, and cause alveoli crashing when they cross a threshold

At this point, SnC come into the picture. SnC slow down the rate of stem cell renewal p, because of their secreted protein profile (SASP). For example, SASP contains proteins that bind and inactivates an important growth factor for stem cells, IGF1. Thus, there is a critical SnC level where p drops below d1, at which both S and D crash. We will assume here that the main factor is SASP coming in the circulation from all the SnC in the body. Local SnC in the alveoli probably also play a role.

Suppose the proliferation rate of S drops with SnC level X, such that $p = p(X)$. Thus, p reaches its critical value p=d1 when X reaches a threshold $X_{c,IPF}$ (Fig 7.13). In most people, $X_{c,IPF}$ is high so that X never reaches it. Unfortunately, some people are susceptible, and have a lower $X_{c,IPF}$, as we will discuss below. In youth, X starts low, and rises over the decades. When X approaches $X_{c,IPF}$, S cells rise causing fibrosis and eventually tissue collapse. As we will see in the next lecture, once fibrosis is activated, it is generally irreversible and matures over several months. Thus, IPF incidence is akin

Figure 7.13

Figure 7.14

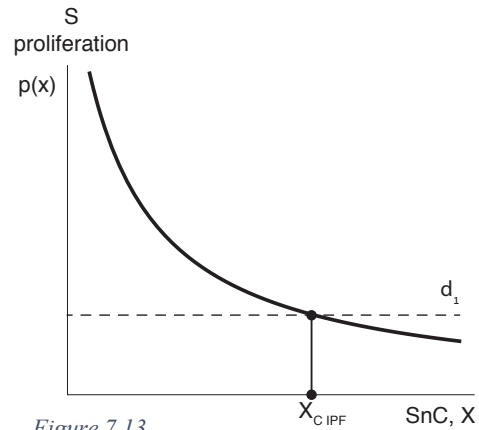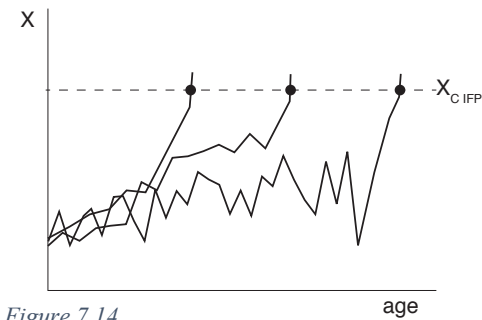to the first-passage time to the threshold $X_{c,IPF}$. Thus, in this picture, onset of IPF is a threshold-crossing phenomenon.

To calculate the incidence, which is the probability of crossing the threshold at a given age, the math is the same as in the previous lecture (Fig 7.14). The threshold $X_{c,IPF}$ replaces the death threshold Xc. The hard work we did in the last lecture pays off! The incidence of the disease follows a Gompertz-like law, with exponent $\alpha = \eta \, (X_{c,IPF} + k)/\epsilon$. Incidence is thus exponential with age (Fig 7.15). The observed slope of the incidence curve suggests that $X_{c,IPF}$ is

Figure 7.15
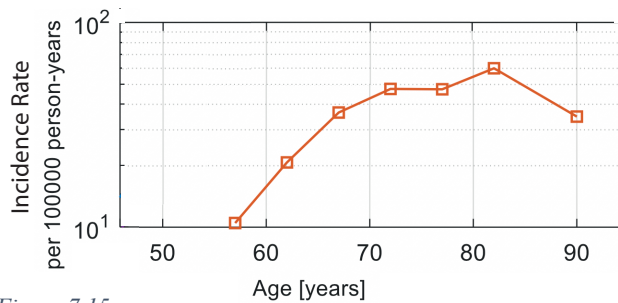
about 50% of the threshold Xc for mortality in the previous lecture.

What explains the drop in incidence at old ages? One simple reason is that at old ages, everyone who is susceptible has already gotten sick, and there few susceptible people left. Let's understand the susceptibility to this disease.

Susceptibility to IPF involves genetic and environmental factors that increase stem cell death

Who is susceptible? Those with a particularly low threshold, $X_{c,IPF}$, smaller than the threshold for mortality X_c. To understand this, we can examine the genetic risk factors for IPF. Many IPF cases cluster in families (estimated at 15% of the cases). First-degree relatives of a patient have a 5-fold higher risk of contracting IPF. The gene variants in these families have been studied extensively.

There are two classes of gene variants that increase the risk of IPF. The first class is in the surfactant genes expressed by S cells. These variants produce unfolded surfactant proteins that damage S cells and increase S cell death, d1. Furthermore, since surfactant is protective, reduced surfactant may also increase cell death rates. Increasing death rate d1 lowers the IPF threshold $X_{c,IPF}$ (Fig 7.16). Thus, these gene variants act to decrease the IPF threshold, making the disease much more likely.
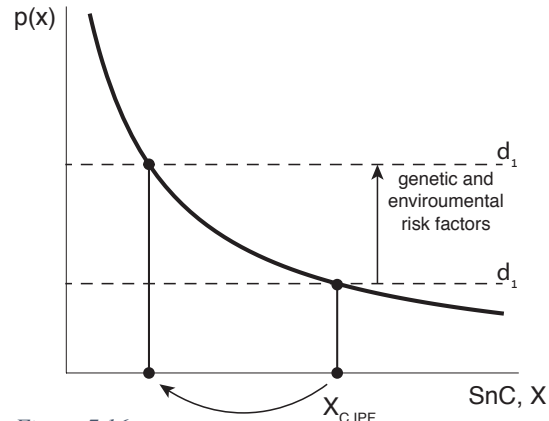

Figure 7.16

The other class of variants also affects S cells. These are **telomerase** genes. Telomerase is important to allow stem cells to divide many times. In each cell division, the DNA ends called **telomeres** become shorter. When DNA becomes too short, the cell stops dividing and becomes senescent- this is how SnC were first discovered by Hayflick in the 1960s, a process now called replicative senescence. Stem cells have an enzyme called telomerase that adds back the missing DNA ends allowing stem cells to divide indefinitely. Thus, the telomerase variants reduce S cell proliferation rate p and increase their death rate d_1 (or equivalently their removal by becoming senescent). This also raises the possibility that local senescence in the alveoli might be at play, in addition to the SASP form the systemic SnC throughout the body.

IPF also has environmental risk factors, such as smoking that increases the risk of IPF by 2-fold. Smoking affects rate of local SnC production, and also increases the death rates d1 and d2. Exposure to toxins such as asbestos also increases d_1 and d_2.

Thus, both the genetic and environmental factors tend to lower $X_{c,IPF}$ and increase the risk of IPF.

To sum up, homeostasis of labile tissues with stem cells at the front line, such as the alveoli, requires feedback regulation between stem and differentiated cells. This feedback is fragile to a reduction in stem cell proliferation. As proliferation drops towards stem cell death rate, the fraction of stem cells in the tissue rises and sets off irreversible fibrosis. Such a reduction in proliferation is caused by SnC that accumulate with age. The statistics of SnC fluctuations explain the exponential rise of IPF incidence with age. The drop at old ages occurs as all those susceptible due to genetic and environmental factors have already got the disease.

IPF may be mathematically analogous to another age-related disease, osteoarthritis.

The analysis of IPF raises the possibility that the exponential incidence of other age-related diseases might also be caused by a threshold crossing of SnC. To make progress, we need

to analyze each disease and understand its biology. It is likely that there will be several classes of diseases with different reasons for the threshold.

An age-related disease that might be in the same class as IPF is **osteoarthritis**. Osteoarthritis is a very common condition that occurs in about 10% of those over 60, in which the protective cartilage that cushions the ends of the bones wears down over time. Although **osteoarthritis (OA)** can damage any joint, the disorder most commonly affects joints in hands, knees, hips and spine. The main symptoms are pain and stiffness in the joints. It is a progressive disease with no cure, except joint replacement surgery.

The joint is made of tough fibrous cartilage. The business end of the cartilage is the very smooth edge region where two parts of the joins meet. This is the front line of the tissue, and where the wear-and-tear occurs. The cartilage is constantly remodeled by chondrocytes D that make the fibers for strength and elasticity, including collagen 2. These D cells are generated by stem cells S called progenitor cells. The stem cells in the joint are at the front line, just like in the alveoli. The reason is that cells have a hard time moving through the cartilage, and thus S cells need to be where new D cells are needed, namely at the front line.

The joints suffer a lot of mechanical stress, especially in regions that support the body's weight. In the young, this stress doesn't do much and the joints are fine for 50 or more years. But at old ages, OA can set in. In a process that takes a few years, cartilage is lost, D cell number reduces, and the fraction of S cells increases. The S cells make tougher fibers than in normal cartilage, such as collagen 1 instead of collagen 2, making the tissue stiffer and less elastic [https://link.springer.com/chapter/10.1007/978-3-319-53316-2_3]. As a result, cracks form, leading to a hole that goes right down to the bone. This hole occurs in the part of the joint that bears the most weight, and thus has the highest cell death rates (Fig. 7.17).
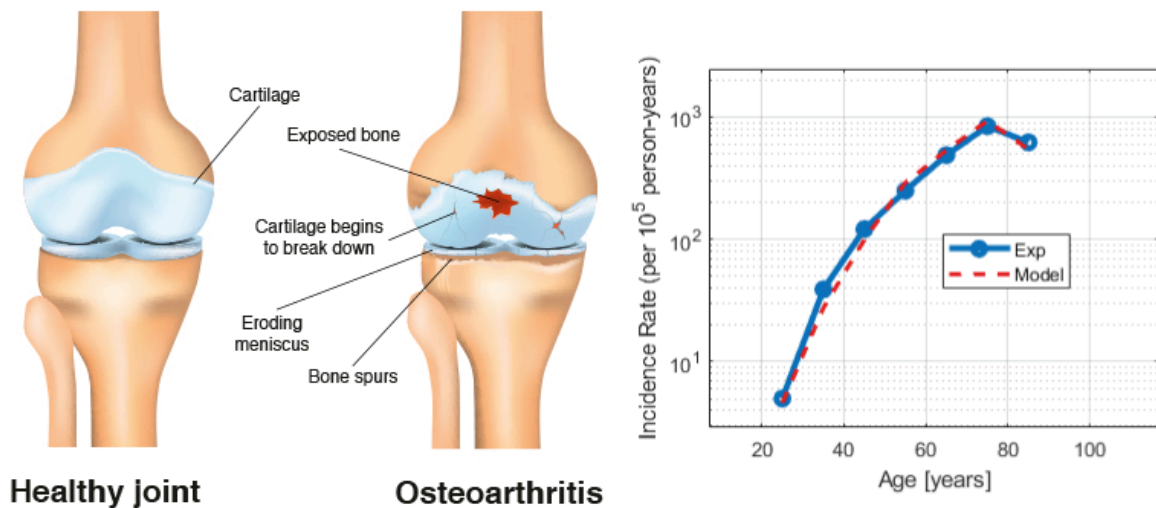

Figure 7.17

Thus, the two diseases have a **mathematical analogy.** The death rate of both stem and differentiated cells is high because both are at the front line. The death rate varies across the tissue and is highest where the most pressure occurs. Reducing the proliferation rate of S cells down towards their death rate leads to a rise in the stem cell fraction S/D, secreting more collagen 1, and eventually the cells are lost altogether. This reduction in S proliferation can be caused by SASP secreted by the SnC in the body, as well as local SnC

in the joint. Indeed, removing SnC alleviates OA in mice models. This picture thus suggests that OA occurs at a SnC threshold $X_{c,OA}$.

Susceptibility to OA means a low threshold $X_{c,OA}$, as in IPF. Such a low threshold can be due to genetic and environmental factors. Environmental risk-factors for OA include being overweight, which increases the load on the joints and hence increases death rates and lowers the threshold. Genetic factors are also important, as OA has about a 50% heritability. Affected genes include fiber components like certain collagens (including collagen 2) and other cartilage components, as well gene variants for the signaling molecules IGF1 and TGFbeta relevant to the feedback circuit [https://doi.org/10.1016/j.joca.2003.09.005].

It is fun to think that diseases as different as a lung disease and a knee disease might have common fundamental origins.

Other age-related diseases show diverse links with SnC and age

Most age-related diseases tested so far are alleviated by removing SnC in mice. These diseases might connect to SnC in different ways. Consider for example, the exponentially rising risk of death from infection. One cause can be the saturation of NK cells by the need to remove the rising SnC levels that occur with age. This saturation can prevent NK cells from carrying out their role in fighting infection, namely killing virally-infected cells. The immune system generally shifts with age towards innate immunity (e.g. macrophages) and away from adaptive immune system (T-cells). This 'myeloid' shift further impairs the ability to combat viruses and bacteria.

The saturation of NK cells also impairs their ability to kill cancer cells. Most cancers are age-related diseases. A classic explanation for the age-dependence of cancer is the need for several mutations in the same cell. Most cancers require a series of mutations to knock out multiple pathways that prevent the cell from mutating rapidly and growing out of control. Such a multiple-hit process has a likelihood that rises roughly as the age to the power of the number of mutations. Cancer in the young often occurs because one of the mutations is already present in the germline and thus in all cells of the body. Other mechanisms can also be at play: rising SnC levels cause chronic inflammation, which is thought to give a growth advantage to certain mutant stem cells on their way to becoming cancerous.

Finally, type-2 diabetes (T2D) is an age-related disease that has a threshold-like mechanism. Late-stage T2D involves loss of beta cells when the glucotoxicity threshold is crossed, as we saw in lecture 2. With age, the proliferation rate of beta cell goes down, and this threshold drops lower and lower. Genetic factors that affect glucotoxicity can further lower the threshold. Inflammation due to SnC causes insulin resistance, as do lack of exercise and obesity. Insulin resistance raises blood glucose levels faster than beta cells can proliferate and compensate, increasing the risk of crossing the threshold for T2D.

A grand project is thus to find mathematical classes of diseases, to understand the ways in which disease incidence rises with age, and to provide clues for treatment.

Exercises:

7.1 Stem cell feedback that keeps constant S: Consider the following feedback loop in a labile tissue. Both stem cells and D cells secrete factors that increase differentiation rate. The differentiation rate is $q(S, D) = q_0 SD$.
(a) Write down the equations for this circuit.
(b) Simulate this circuit (or use linear stability analysis) and test whether the steady-state is stable.
(c) Show that the steady-state concentration of S cells is independent on S proliferation, p.
(d) What is the concentration of D cells as a function of p?
(e) Is the effect of this feedback biologically useful?

7.2 Oscillations in a labile tissue circuit: consider a feedback loop with a single interaction in which D increases differentiation rate $q(S, D) = q_0 SD$.
(a) Write the equations and simulate them.
(b) Explain the resulting oscillations in S and D numbers intuitively.
(c) Read about the predator-prey model in ecology called the **Lotka-Volterra** model. What is the analogy?
(d) Why are ecology population models for species population an interesting resource for modelling cell circuits?

7.3 Labile tissue with protected stem cells: Consider a tissue in which the stem cell death rate d1 is negligible, whereas the D cells have a sizable death rate d2.
(a) Suppose that a feedback loop provides a stable-steady state. What happens to the S/D ratio as S proliferation p is lowered? Is there a point of collapse?
(b) What diseases might characterize such tissues, more often than tissues with stem cells at the front line (high d1)?
(c) Design a feedback loop that provides D levels that are insensitive to variations in stem-cell proliferation p.

7.4 NK cell homeostasis circuit: NK cells are constantly produced by stem cells in the bone marrow. They have a high death rate d2, unless they go into the bodies tissues and find cells that make a survival signal (IL15-IL15R). Most cells of the body produce this survival signal. When NK cells touch the donor cells, they receive the signal, and their death rate drops to zero. NK cells constantly patrol the body and go into and out of the blood stream.
(a) Write equations for NK cell numbers.
(b) What determines the NK cell lifetime of about a week in humans?
(c) NK cells were introduced into a mouse mutant that cannot produce its own NK cells. These cells lasted for at least six months. Explain this result.
(d) Explain how this homeostasis mechanism ensures that the number of NK cells matches the number of cells in the tissues that need NK cell surveillance.

7.5 Stem cell symmetric and asymmetric divisions: Consider the case where a stem cell can divide to form either two stem cells or two differentiated cells, 2S or 2D. This is called symmetric division. Asymmetric division is the case where there is also a third possibility, of dividing to form one D and one S cell.

(a) What is the difference in the mathematical equations for the S and D populations in the two cases?

(b) How does this affect the S/D ratio as proliferation p approaches death d_1?

7.6 Two disease thresholds: Consider two age-related diseases with SnC thresholds Xc,1 and Xc,2. Suppose the two diseases can occur in the same person. What would you expect about the relative timing of the diseases in the same person? How would you test this hypothesis? What are some confounding factors?

7.7 OA regions: Explain why osteoarthritis occurs in certain regions of the joint. In the hip it occurs in the top part of the joint. In the knee it occurs at the inside rim in people with legs oriented slightly as an X-shape, and at the outside rim of the knee in people with a bowlegged, O-shaped configuration.

7.8 Death rates: In healthy alveoli tissue there are approximately twice as many AT2 cells (S) than AT1 cells (D). Since S cells are smaller they make up only 7% of the surface area *Am Rev Respir Dis*. 1982). Estimate using the simple calculations in the lecture what is the ratio between S proliferation and death rates. In the knee joint, progenitor cells (S) amount to about 4% of the total cell population, rising to about 8% in OA. What is the ratio of proliferation to death rates?