How does an individual tumor develop?
Tumor development is a multi-step process in multiple distinct epithelial tissues.

- **Colon**:
  - Normal: 5-20 years
  - Initiated: adenoma
  - Pre-cancer: dysplastic oral leukoplakia
  - Cancer: 5-15 years

- **Head and Neck**:
  - Normal: tobacco use 4-10 years
  - Pre-cancer: dysplastic oral leukoplakia
  - Cancer: 6-8 years

- **Cervix**:
  - Normal: CIN 1
  - Pre-cancer: CIN 3/CIS
  - Cancer: 10-20 years

- **Lung (Smokers)**:
  - Normal: 20-40 pack-years
  - Pre-cancer: atypical hyperplasia
  - Cancer: DCIS
  - Latent cancer: 6-10 years

- **Breast**:
  - Normal: atypical hyperplasia
  - Pre-cancer: PIN
  - Cancer: ≥10 years

- **Prostate**:
  - Normal: 20 years
  - Pre-cancer: PIN
  - Cancer: 3-15 years

Figure 11.8a The Biology of Cancer (© Garland Science 2014)
Colorectal carcinoma.  (Vogelstein et al 1989)
Molecular origins of cancer: Molecular basis of colorectal cancer.

Markowitz SD¹, Bertagnolli MM.

How important are non-genetic programs in determining the biology of cancer cells and thus tumors?
Can we understand the pathogenesis of a tumor in terms of the somatic mutations that it accumulates in the genome of its neoplastic cells? e.g., pancreatic adenocarcinomas
This scheme allows us to understand, at least in outline how a primary tumor can be formed.
Multiple subcircuits within a human cell must be perturbed before (experimental) transformation of human cells succeeds.
Why is human cell transformation so complicated?

~$10^{11}$ cell divisions

~$10^9$ cell divisions?

~$10^{19}$ cell divisions?

If the risk of somatic mutations is proportional to the cumulative number of cell divisions in a lifespan then the cells of larger, long-lived mammals must have acquired proportionally increased numbers of anti-neoplastic defenses.

If we consider the risk of somatic mutations, the cells of larger, long-lived mammals have acquired proportionally increased numbers of anti-neoplastic defenses.
Can we understand the pathogenesis of a tumor in terms of the **somatic mutations** that it accumulates in the genome of its neoplastic cells?

Colorectal carcinoma. (Vogelstein et al 1989)

Figure 11.10  *The Biology of Cancer* (© Garland Science 2007)
A major factor: The continuing influence of the differentiation program of the cell-of-origin

Two genetically identical tumors from closely related cell types

SCLC = small cell lung cancer

Differential gene expression in CMV TKO NFIB\textsuperscript{low} tumors and CGRP TKO tumors (\(|\text{fold change}| > 2\), adj. \(P < 0.05\))

Different genes are up/down-regulated in SCLC cells originating from \textbf{two distinct subtypes} of neuroendocrine cells in the lungs.

\begin{itemize}
\item \textbf{CMV} \textbf{NFIB}^\text{low} primary tumors \((N = 9)\):
\begin{itemize}
\item 978 Higher
\item 1138 Higher
\end{itemize}
\item \textbf{CGRP} \textbf{NFIB}^\text{high} primary tumors \((N = 12)\):
\begin{itemize}
\item 492 Down
\item 1251 Up
\item 0 Down
\item 4 Up
\end{itemize}
\end{itemize}

\textbf{Differences in transcriptomes}

The continuing influence on transcriptome of the differentiation program of the normal cell-of-origin
The transcriptome of the normal cell-of-origin continues to imprint itself on the behavior of derived neoplastic cells.

The acquisition of somatic mutations does not eradicate the continuing influence of the normal cell-of-origin.
Figure 11.10  The Biology of Cancer (© Garland Science 2007)
Figure 11.10  The Biology of Cancer (© Garland Science 2007)

The “invasion-metastasis cascade”

What happens here?
The "invasion-metastasis cascade"

What happens here?

How do cancer cells acquire all of these distinct capabilities? Are additional somatic mutations required?
How do cancer cells acquire all of these capabilities?

A key clue: The behavior of a BPLER br. ca. xenograft in mouse host

Implanted (human) cytokeratin-positive cancer cells (therefore epithelial)

human vimentin-positive (therefore mesenchymal) cancer cells of human origin

Invasive cell

recruited mouse stroma

K. Hartwell and T. Ince
Contextual signals influence the induction of EMT programs

**EMT** = epithelial-mesenchymal transition

BPLER tx human mammary epithelial cells in mouse host

Border cells **lose** epithelial characteristics

Border cells **gain** mesenchymal characteristics

**cytokeratins** (epithelial)  
**human vimentin** (mesenchymal)
How do carcinoma cells acquire traits needed to metastasize? **One possible solution:**

The epithelial-mesenchymal transition (EMT) is a complex, multi-faceted program involving multiple coordinated changes in cell-biological properties.

For both normal and neoplastic epithelial cells
There are many alternative EMT programs that share in common a relatively small set of cell-biological changes.

The canonical EMT program

- Cobblestone to Elongated
- Non-mobile to Mobile
- Non-invasive to Invasive
- Polarised to Non-polarised

Epithelial markers:
- E-cadherin
- Desmoplakin
- Keratin

Mesenchymal markers:
- N-cadherin
- Vimentin
- Fibronectin
A group of pleiotropically acting transcription factors (EMT-TFs) that induce EMT at various stages of metazoan embryogenesis.

EMT = epithelial-to-mesenchymal transition
Discovery of tumor-initiating cells (TICs) (=cancer stem cells) (CSCs)

Fractionate subpopulations of carcinoma cells from a single human breast cancer

al-Hajj et al. 2004

Figure 11.14a The Biology of Cancer (© Garland Science 2007)
Shifting gears: Is there any connection between the EMT & SC programs??

Immortalized human mammary epithelial cells

\[ \text{CD44}^{\text{hi}}/\text{CD24}^{\text{lo}} \] (position of stem cells)

\[ \text{CD44}^{\text{lo}}/\text{CD24}^{\text{hi}} \] (position of non-stem cells)

Al-Hajj & Clarke, 2004 (in breast cancer cells)
Induction of EMT by Snail and Twist EMT-inducing TFs generates CD44\textsuperscript{hi} CD24\textsuperscript{lo} cells including CSCs.

CD44\textsuperscript{lo}/CD24\textsuperscript{hi} (position of non-stem cells)

CD44\textsuperscript{hi}/CD24\textsuperscript{lo} (position of stem cells)

Morphological shift in monolayer culture

S.A. Mani & W. Guo

epithelial \rightarrow mesenchymal
Can expression of EMT-TFs in fully normal mammary epithelial cells induce the formation of normal mammary stem cells?

Transient Expression (4-5 days) of two EMT-inducing transcription factors (Slug + Sox9) prior to fat pad implantation induces a >100-fold excess of normal mammary stem cells (visualized 6 weeks later).

Concomitant transiently induced expression of Slug + Sox9 EMT-TFs

Wenjun Guo
CD44 high

CD44 low

EMT program does not operate as a binary switch

EMT program
Invasiveness
Motility
Metastatic dissemination
Suppressed apoptosis
Resistance to chemotherapy
Stemness

E-cadherin

fibronectin

p63

CSCs

Epithelial

Mesenchymal

Brian Bierie
CD44^low\quad CD44^\text{high}

Cancer stem cells (CSCs, TICs)

E-cadherin

vimentin

ITGB4

fibronectin

CSCs

qM cells

quasi-mesenchymal

Epithelial

Mesenchymal

Brian Bierie
What if we developed an anti-CSC treatment?

Ideally:

**before treatment**
- cancer stem cells
  - transit-amplifying cells
  - etc.

**effects of treatment**
- transit-amplifying cells
  - etc.

**clinical response:**
- cure
Reversibility of SC differentiation

self-renewing stem cell

transit-amplifying cells

post-mitotic differentiated cells
What if we developed an anti-CSC treatment, e.g., cAMP induction?

Ideally:

- Before treatment: cancer stem cells
- Effects of treatment: transit-amplifying cells
- Clinical response: cure

What if this happens instead?

- Before treatment: cancer stem cells
- Effects of treatment: transit-amplifying cells
- Clinical response: relapse after cessation of treatment

Plasticity
Given all this, how does multi-step tumor progression actually proceed?

The Darwinian Model
Intra-tumoral diversification:

The Darwinian Model: Mutations spawn diverse clonal sub-populations more rapidly than selection eliminates them.
However, the Darwinian model does not address the complexity of multiple alternative phenotypic states at each step of tumor progression.
However, this scheme has its flaws:
Which cells are most likely to sustain the mutations that lead to a more advantageous phenotype?

1. The stem cells are relatively small in number. Therefore small target size.
2. The stem cells generally proliferate far less often than do the transit-amplifying/progenitor cells. (Typically the vast bulk of the mitotic activity in a tissue is presented in the transit-amplifying/progenitor compartment. Therefore, far less opportunity for somatic mutations being sustained in the stem cell compartment.

Hence, it is far more likely that the transit-amplifying compartment rather than the same cell compartment is the source of the mutations that generate novel variants.
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If the mutations are sustained in the transit-amplifying compartment, how are they sustained and perpetuated in the descendant population?
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And even if this is true, which cells are the objects of selection?
If the mutations are sustained in the transit-amplifying compartment, how are they sustained and perpetuated in the descendant population?

And even if this is true, which cells are the objects of selection?

(Unlikely to be the stem cells, which lack the display of certain advantageous phenotypes.)
(Unlikely to be the more differentiated cells, unless they can generate less-differentiated cells)
And then there is the question of **intratumoral inter-clonal collaboration**

**Differential response of Epi. and Mes. tumors to checkpoint immunotherapy**

- **Epithelial tumor**
- **Mesenchymal tumor**

- Cell lines: Mixed
- Subcutaneous
- **Anti-CTLA4 (9H10)**
  - 200ug, every 3 days

**Graphs:**
- Tumor size (mm^2) vs. time (Days)
  - Control
  - anti-CTLA4

**Anushka Dongre w. Mohammad Rashidian (Ploegh)**
And then there is the question of **intratumoral inter-clonal collaboration**

Minority (10%) **mesenchymal** subpopulations can protect majority **epithelial** populations from elimination by anti-CTLA4 therapy

Fully mesenchymal

Fully epithelial

9-fold excess of **epithelial** cells over **mesenchymal** cells. (Ratio is conserved in fully formed mixed tumors)

Make mixed tumors

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Anushka Dongre w. Mohammad Rashidian (Ploegh)