SEASON 2

Understanding Cancer

Lecture 12

Types of signalling pathway: normal and dysregulated Notch

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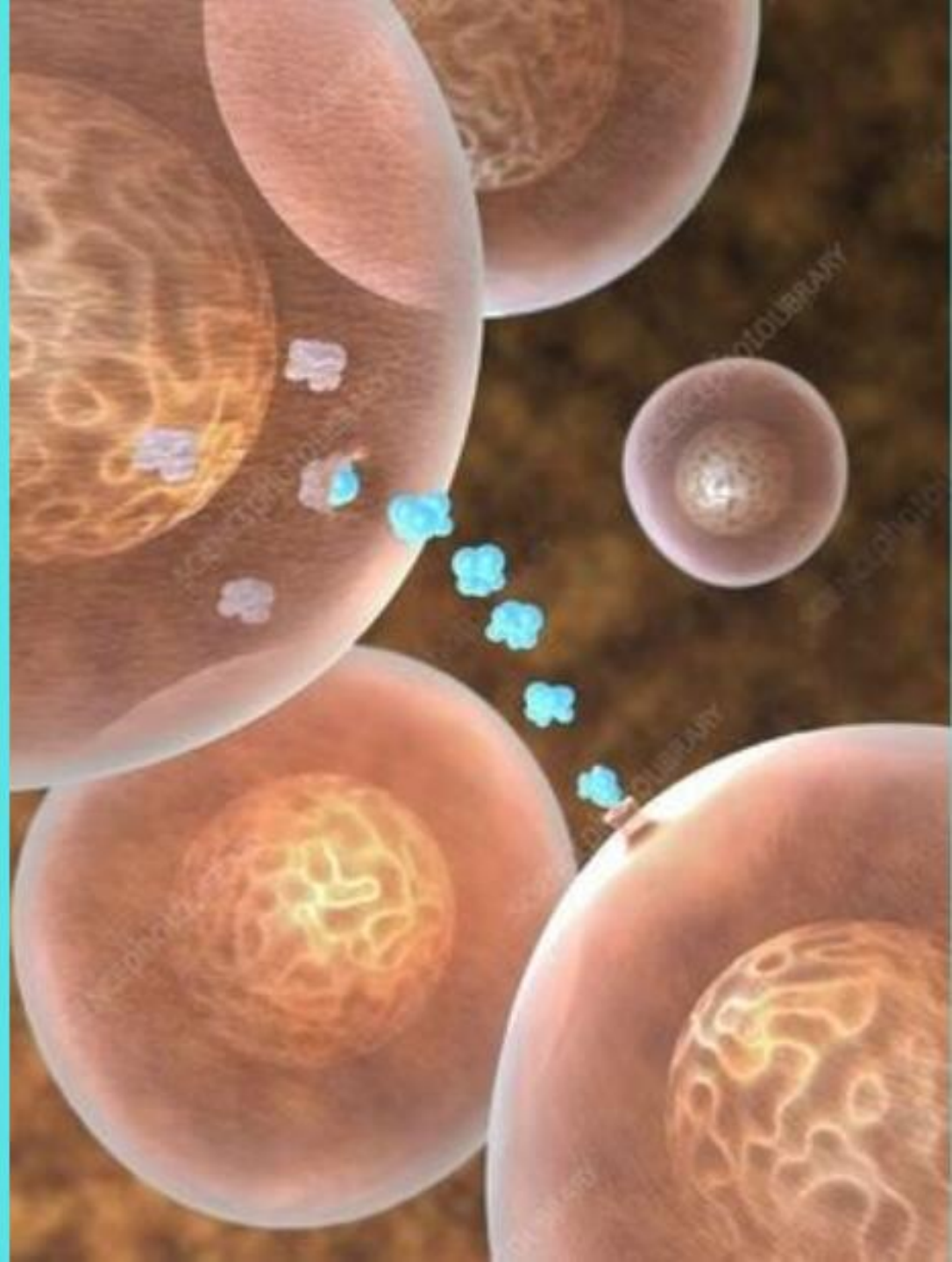

RECAP:

What you hopefully should understand so far from Lecture 11

- **The human Philadelphia (Ph) chromosome arises from a translocation between chromosomes 9 and 22 of the t(9;22). This affects the ABL and BCR genes.**
- **There are two main cancers associated with ABL-BCR translocation: chronic myeloid leukaemia (CML) that mainly affect middle-aged patients and B-cell acute lymphoblastic leukaemia (B-ALL) that affect paediatric patients under 15 years old**
- **Other Abl fusion can occur that can cause T-cell acute lymphoblastic leukemia (T-ALL).**
- **RAS is the main signal transduction pathway linked by BCR-ABL. Upon activation of BCR with GRB2, it recruits SOS ("son-of-sevenless") to stimulate RAS.**
- **RAS can be also activated by inhibiting GAP or activated by CRKL via SOS.**

What will we learn today?

- ***The structure of Notch receptors***
- ***The types of Notch receptors***
- ***Receptor activation: Normal Notch signalling pathway***
- ***Signal transduction: Normal Notch signalling pathway***
- ***Cellular response: Normal Notch signalling pathway***
- ***Examples of cancers where dysregulated Notch signalling pathway***

GENTLE REMINDER
An ideal way of learning:

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday

Mini-lectures.

Approximate total time: 1 hour

Divide over 7 days at your **own pace**.

Challenge yourself with a quiz!

HN

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RECAP: How to support your learning?

- **Key facts with diagrams by HN designs presented in a simplified way.**
- **Glossary to help understand what key words mean.**
- **Summary doodle revision posters by HN designs.**
- **Quizzes to test your knowledge and reflect.**
- **Reference list for further reading.**

Acknowledgements: Special thanks to my parents, family, friends and colleagues for their support and the respected teachers and health professions who taught me and installed the passion of cancer/oncology.

The structure of Notch receptors

The structure of Notch receptors

They are transmembrane proteins expressed on the surface of cell membrane.

They consist of

Extracellular region

- *N-terminal EGF repeats between 29-36*
- *Juxtamembrane Negative regulatory region (NRR):*

It consists of two divisions:

Three Lin-12/Notch repeat (LNR)

Heterodimerization domain (HD)

Transmembrane domain

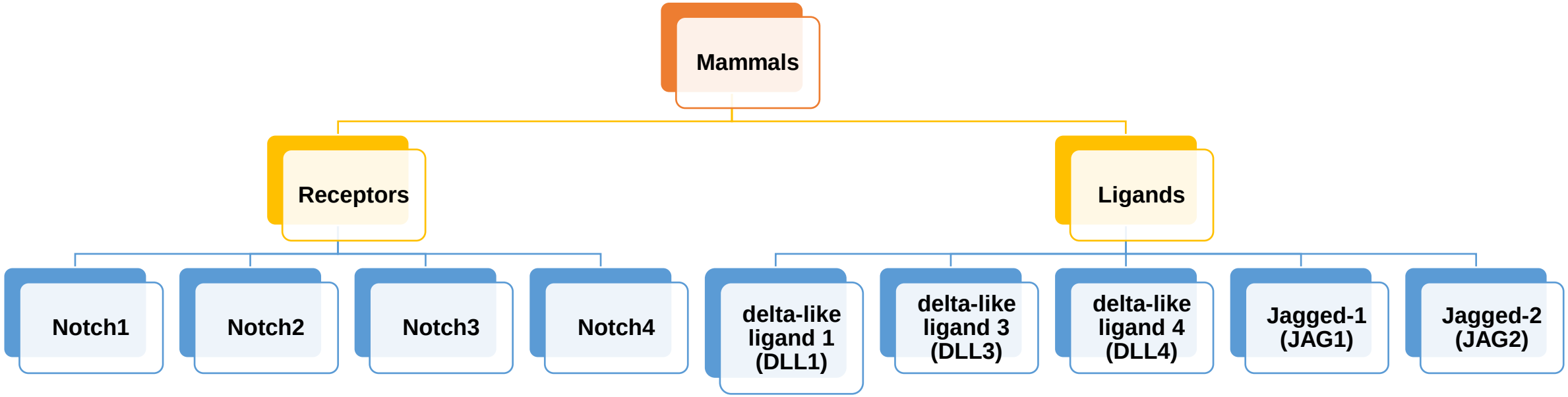
Intracellular region

- *Protein-binding RAM region*
- *Seven ankyrin (ANK) repeat domains*
- *Transcriptional activation domain (TAD)*
- *C-terminal degron domain with high levels of the amino acids proline, glutamate, serine and threonine (PEST)*

(Aster *et al.* 2017; Yuan *et al.* 2015)

The types of Notch receptors

The types of Notch receptors



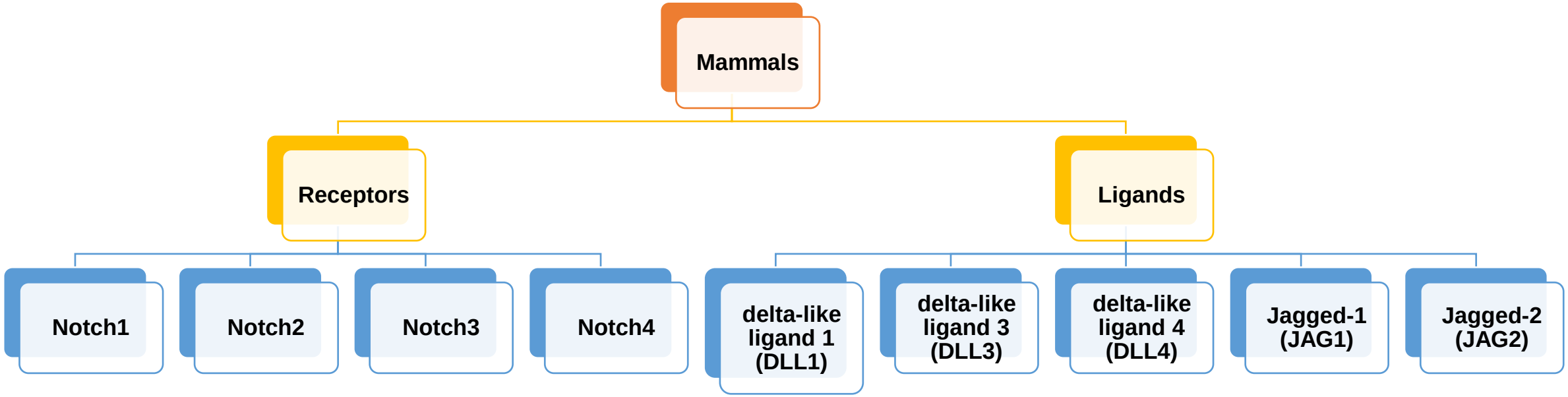
Notch1 and Notch2 are expressed in **tissue development and adult mammals**.

Notch3 are expressed in **vascular smooth muscle and pericytes.**

Notch4 are expressed in **endothelium.**

(Yuan *et al*. 2015)

The types of Notch receptors



DLL1 and DLL4 are **transmembrane proteins and members of the Delta family of ligands.**

DLL4 **cannot activate Notch receptors and encodes a decoy receptor.**

JAG1 and JAG2 are members of **Serrate family of ligands.**

(Yuan *et al*. 2015)

Receptor activation

Receptor activation

The ligand binds to the Notch receptor.

Depends on cleavage by three enzymes

1

- **Furin-like protease.**
- **Location: Cleaves at site S1 in the heterodimerization domain**
- **Function: Creates a heterodimer made of Notch extracellular subunit and a transmembrane Notch subunit.**
- **It is held off state by NRR.**

2

- **ADAM disintegrin and metalloproteases family predominantly ADAM17 or ADAM10**
- **Location: Cleaves at site S2 outside the transmembrane domain.**
- **Following the binding of Notch to its ligand, autoinhibitory effect of NRR takes place, ADAM cleaves.**

3

- **Two E3 ligases: Neuralized and Mind Bomb**
- **Function: ubiquitination of ligand cytoplasm.**

(Aster *et al.* 2017)

Signal transduction

Signal transduction

An enzyme called presenilin-dependent gamma secretase releases the Notch intracellular domain (NICD) of the activated receptor and translocates it into the nucleus.

In the nucleus, the NICD binds to the ubiquitous transcription factor that can repress transcription called CSL.

CSL is an abbreviation for CBF-1/suppressor of hairless/Lag1.

Together, a NICD-CSL complex.

The NICD-CSL complex is converted to an activator for the transcription of Notch target genes.

Other ways of activating transcription for is binding with the DNA sequence and the SMART complex in absence of NICD.

(Yuan *et al*. 2015)

Cellular response

Target genes

NICD-CSL complex in the nucleus

❑ **p27cip1/waf1**

- Inhibited cyclin dependent kinase 2 (cdk2) activity and halted cell cycle
- G1 phase of the cell cycle by p27(Kip1)
- G2 phase by p21(Waf1)

❑ **Cyclin D1** cell cycle regulation

❑ **c-Myc** - growth and cell cycle regulation

❑ **p21**

- Inhibitor of cyclin E/cdk2.
- Its expression regulated by tumour suppressor p53 and oncogene K-ras.

(Schmidt *et al*. 2001; Hayat, 2005)

Target genes

NICD-CSL complex in the nucleus

❑ **Survivin**

- A member of Inhibitors of apoptosis (IAPS)

❑ **Slug**

- Regulates Epithelial–mesenchymal transition (EMT) and is involved in invasion and evading apoptosis.

❑ **Nanog**

- Stem cell differentiation where cells are pluripotent.
- Pluripotency - they can become any cell type.

❑ **Nuclear factor-kappa B (NF-κB) pathway – later discussed in the series.**

(Shih and Yang, 2011; DeBerardinis and Thompson, 2008; Baker, 2009)

Target genes

CSL-DNA sequence-SMART complex in the nucleus

- Hes (Hairy Enhance of Split)

Cell fate decisions during tissue development.

- Hey (Hairy/Enhancer of Spit related with YRPW motif)

Hey2 decreases skeletal mass and regulates bone remodeling.

Example of tissue: heart

Development and maturation of heart cells in the chambers of the heart and blood vessels (arterial and ventricular cardiomyocytes) and the inner layer of the heart (endocardium)

(Piscione *et al.* 2004; Zanotti and Canalis, 2013; Yuan *et al.* 2015)

Overview of cellular responses

Cell proliferation

Cell differentiation

Homeostasis

Embryo development

Apoptosis

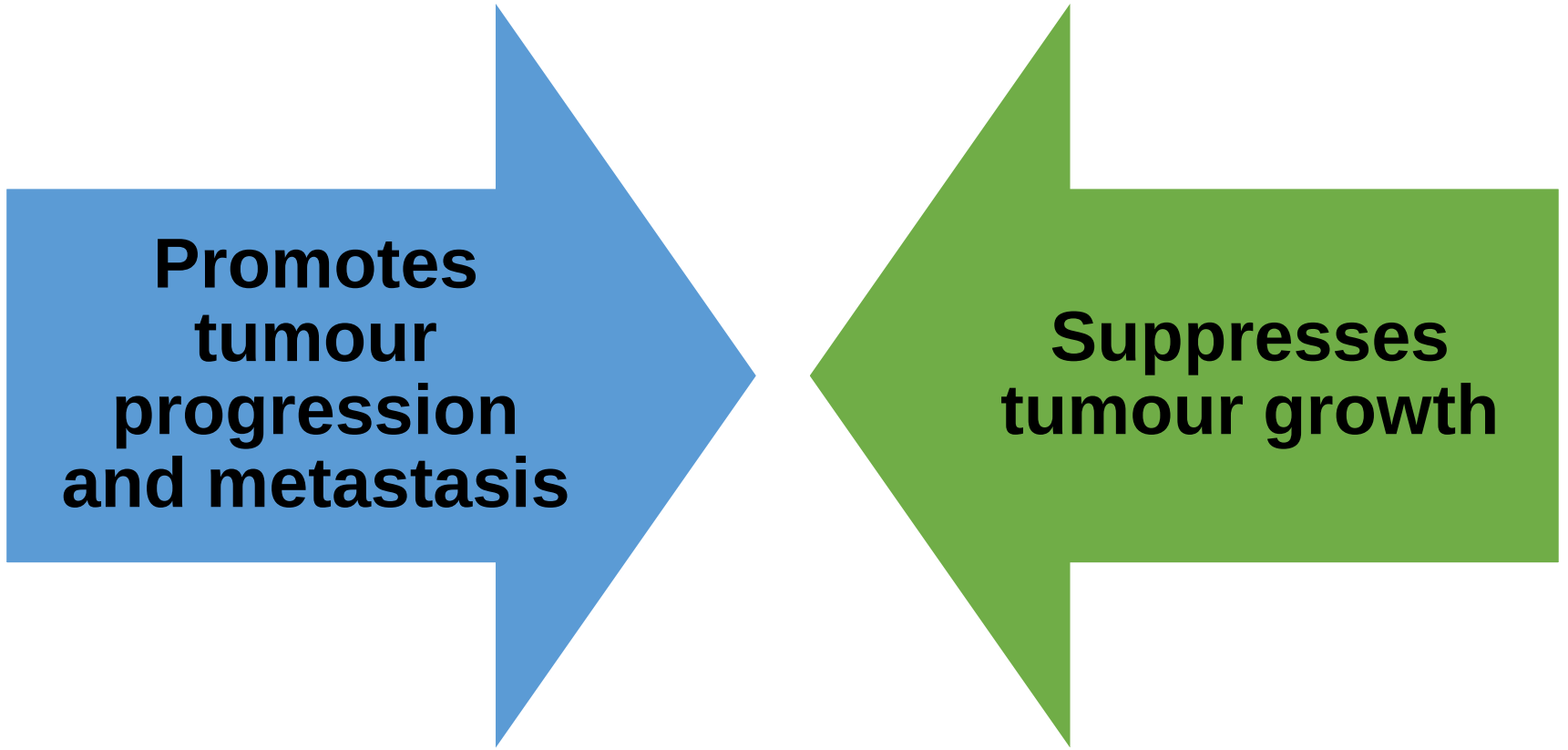
(Yuan *et al*. 2015)

Examples of cancers where dysregulated Notch signalling pathway

Examples of cancers

Liver	**Small cell lung cancer**	**T-cell acute lymphoblastic leukemia (T-ALL)**
Breast	**Prostate**	**Colorectal**
Central Nervous System	**Throat cancer**	**Ewing sarcoma (bone cancer)**

Notch


Promotes tumour progression and metastasis
Suppresses tumour growth

Promotes tumour progression

Mutations in the PEST domains of Notch1, Notch2 and Notch3 dysregulated the Notch signaling.

Amplification of wild-type Notch receptors and ligands.

Notch4 and Notch ligands genes were mutated at low levels.

This increased the expression of Notch target genes which increased the progression of cancer.

(Aster *et al.* 2017; Yuan *et al*. 2015)

Promotes tumour progression

- **DLL4-Notch regulates NF-κB signalling in liver metastasis of human small cell lung cancer (SCLC).**
- **Over 50% of T-ALL tumours have Notch 1 mutations. It stimulated cell proliferation, invasion and chemoresistance.**
- **Notch1 and Notch3 involved in tumour progression and prognosis in Non-small cell lung cancer.**
- **DLL3 and Hes1 overexpression poor survival.**

(Aster *et al.* 2017; Yuan *et al*. 2015)

Suppresses tumour progression

- **Notch suppresses SIRT1 and activates the tumour suppressor p53 to induce growth arrest in B-cell tumours and Ewing sarcoma.**
- **Low expression of DLL4-Notch decreased angiogenesis and tumour growth.**

(Aster *et al.* 2017; Yuan *et al*. 2015)

By the end of this lecture, you should understand

- **Notch are transmembrane protein receptors.**
- **Four receptors found in mammals: Notch1, Notch2, Notch3 and Notch4.**
- **The ligand-receptor complex can be cleaved by three enzymes: Furin-like protease, ADAM family and E3 ligases.**
- **The intracellular domain of the activated receptor is released and can bind to CSL to mediate transcription in the nucleus. CSL can also bind without the intracellular domain to induce transcription.**
- **Notch can promote tumour progression and can also suppress.**

Reference list for further reading

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SEASON 2

Understanding Cancer

Lecture 13

Types of signalling pathway: normal and dysregulated Wnt

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