Necroptosis

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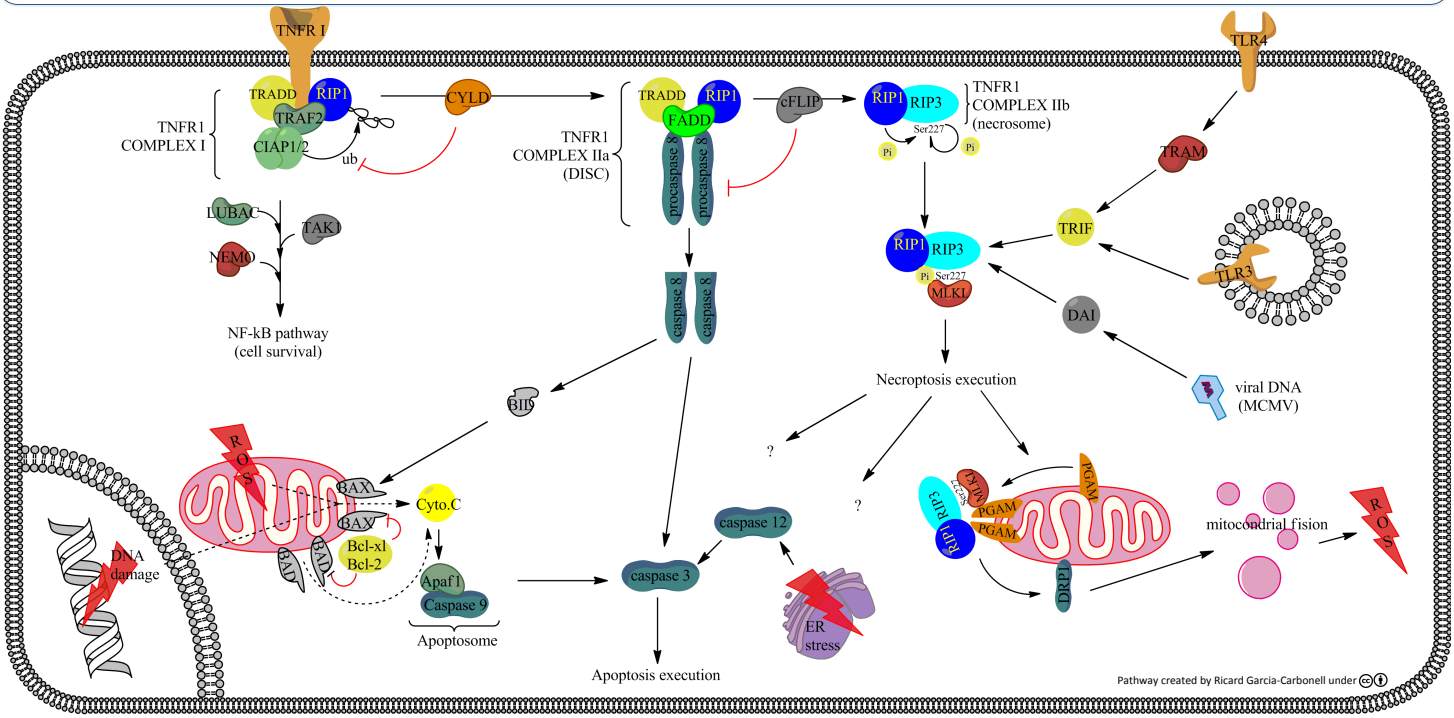
Introduction

Cell survival has been the subject of active research for several years, which has also included cell death, as it is an important mechanism for tissue homeostasis. Historically, it has been described a caspase-driven programed cell death named as apoptosis, and a "non programed cell death", necrosis. However, recent studies indicate that necrosis can also be driven by different stimulus through a programed pathway; therefore a new term has been introduced: necroptosis. It refers to a cell-death receptor-induced, caspase independent, highly regulated type of programed cell death process with morphological resemblance to necrosis. Unfortunately, whereas apoptosis has been largely studied, the molecular composition of the necroptotic pathway has remained elusive until now, as nowadays it is still hard to visually distinguish it

Aims and methodology

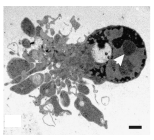
The aim of this project was to unify in a pathway all the different information about necroptosis to to give some light both on how necroptosis is driven and also how we are able to detect it on tissues in order to allow further investigations take in consideration this pathway as it can be of special interest for cancer therapies. This was performed : searching at Pubmed for necroptotic papers latter the literature and product companies for different techniques that allow its differentiation

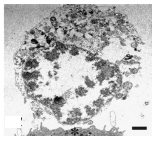


How to distinguish between apoptosis and necroptosis

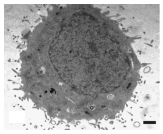
Nowadays, the gold-standard way to distinguish between apoptosis and necroptosis is electronic microscopy

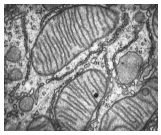
Apoptosis	Necroptosis
Membrane bebbling without loss of membrane integrity	Loss of membrane integrity
Aggregation and fragmentation of the chromatin	No chromatin fragmentation
Cytoplasm condensation and nuclear collapse	Mitochondrial and cytoplasm swallowing
At the end, cellular small bodies are formed	Ends with cellular lysis
Apoptotic bodies formation	No vesicles are formed
Mitochondrial permeabilization	Swallowing induced organelles fision
Less inflammation associated response	Inflammation associated response
Possible activation of caspase pathway Loss of membrane asymmetry	Inhibition of caspases is required


Apoptotic cell[1]


Necroptotic cell[1]


Electronic density changes within apoptotic cell[1]


Healthy cell[2]


Healthy mitochondria[1]


Mitochondrial swelling[1]

Apoptosis or necroptosis?

Different approaches can be performed in order to distinguish between them.

	Apoptois	Necroptosis
Tunel	Clearly positive stained cells	Pale stained cells
Cleaved caspases:		
3	Panapoptotic marker	Negative cells
8	Extrinsic pathway marker	
9	Intrinsic pathway marker	
Annexin V	Positive cells due to asymmetric membrane rupture	Negative cells
Propidium iodae(PI)	Negative cells	Positive nuclear staining
Hematoxilina i eosina	Collapsed cells	Not distinguishable
Electronic microscopy	Major changes involving mitochondria and nucleus	

Conclusions

This work shows a novel pathway that has remained unstudied and classified as a "non-regulated cell death". Weather necrosis is just triggered by the above-mentioned signaling or also by other molecules it is still unknown but this pathway supports the idea that every cell outcome is due to a complex signaling events in a finally balanced but still unknown state.
In order to sum up, a caspase independent program, named necroptosis, in which RIP1, RIP3 and MLKL play an important role can also trigger cell death. Because it has been discovered recently, little is known on how it is driven however further investigation should be done, as it can be an important way to fight cancer because it usually express some necroptotic initiator factors that just need a push up to prompt those specific cancer cells towards necroptosis. This work also shows several approaches to detect necroptosis in order to be taken in account in further investigations

References

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