Regulation of Raf-Kinase with consequences on Therapies.

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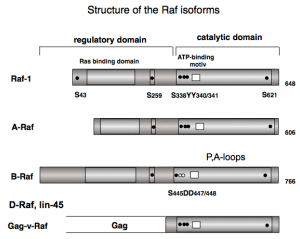
Abstract

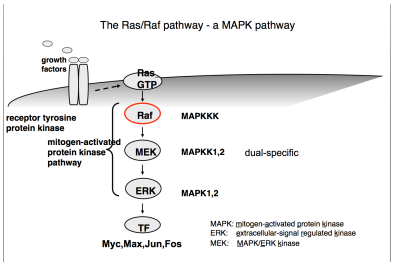
The Raf kinase was discovered 30 years ago as a retroviral oncogene (1). It is a activated in several human cancers and a major target of recent drug design. However, the Raf kinase can also induce differentiation instead of proliferation, depending on the cell-type and growth factor stimuli. Thus an inhibitor of the Raf kinase in cells where it is normally inducing Differentiation, anti-cancer drugs may induce prolieration and cause an undesired opposite effect, increasing or inducing proliferation This has been observed repeatedly in patients treate with one of the novel drugs against the Raf kinase. We also described a negative previously feedback loops inducing upstream signalling to the EGF receptor. Again, inhibition of this loop by drugs against Raf may induce the opposite effect. This was observed and therefore recently a dual therapy was applied in order to compensate for the loss of the negative feedback, with some therapeutic success. Considering the unexpected counterintuitive effects of Raf kinase inhibitors and novel therapeutics it is woth discussing the known regulatory mechanisms we have described, and avoid side-effects.

(1) Moelling et al., Nature 1974; (2)Zimmermann and Moelling, Science 1999; (3) Rommel et al., Science 1999; (4) Zimmermann et al. Oncogene,1997.

Raf kinase signaling

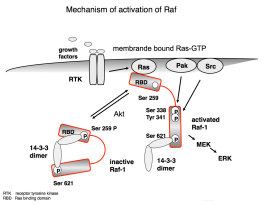


Raf family members

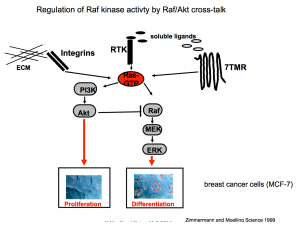


Raf kinase cascade

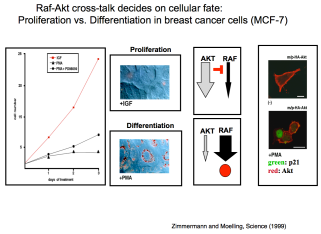
Raf –Akt cross-talk leading to proliferation or differentiation



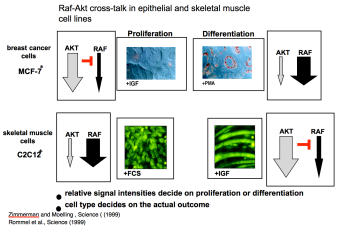
Actiavation of Raf signaling, control by dimer 14-3-3 and P



Akt-Raf signaling pathways with negative regulation of Raf by P of Ser259

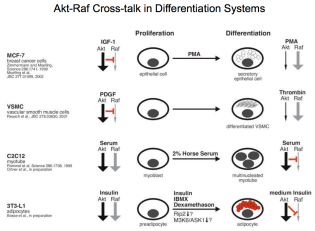


Akt-Raf cross-talk can lead to cell-cycle arrest and differentiation

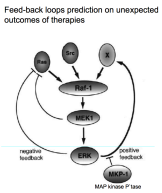


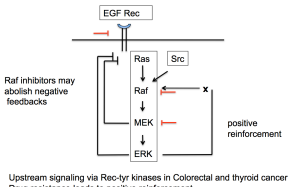
Akt-Raf cross-talk can lead to differentiation and proliferation

Raf- induced cellular responses

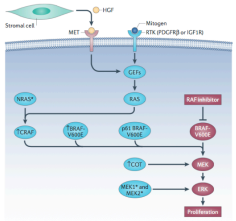


Raf- induced positive and negative feedback loops (upstream signaling)

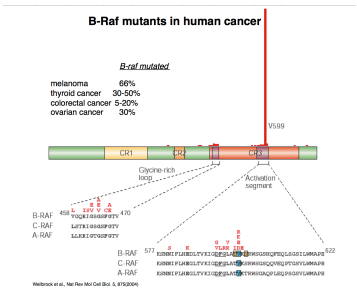




Upstream signaling via Rec-tyr kinases in Colorectal and thyroid cancer
Drug resistance leads to positive reinforcement
Impaired B-Raf kinase is an oncogene driver via Ras



B-Raf mutations in Human cancers with kinase activations



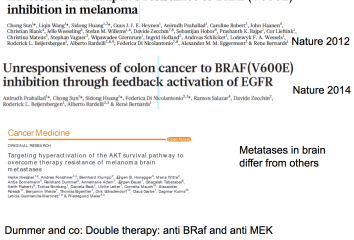
Drugs:

B-Raf inhibitors approved by FDA
Vemurafenib
Dabrafenib
for metastatic and unresectable B-Raf-mutated Malignant Melanoma
ATP competitors Sorafenib approved for renal cell, hepatocellular and thyroid cancers

However: B-Raf mutational status alone does not predict therapeutic response.
Drugs may fail because of B-Raf overexpression or drug resistance can lead to feed back loops.
300 mutations

c-Raf Mutations in genetic disorders
S259 to S259P or S259A
This removes the 14-3-3 linker and partaillly activtes c-Raf, „opens the shell" and allows better binding to RAS-GTP
in Noonan Syndrome
but also in Colon and lung cancer
Overexpressd c-Raf is found in bladder cancer

Drug-induced Raf- mediated upstream signalling required a second drug



Metatases in brain differ from others

Dummer and co: Double therapy: anti BRaf and anti MEK

Summary:

Raf oncogene or differentiation depends on :
Kinases phosphorylation (+/-)
Cross-talk,
signal intensities,
signal duration,
Protein-protein interaction,
tumorsuppressor proteins (Af-6, CNK PDZ Domains)
Scaffolds (dose-dependent)
Feed-back loops
upstream signaling (+/-)

Conclusions

- **Raf crosstalk, induction of differentiation and upstream signalling by negative feed-back loops lead to predicitons of therapeutic side-effects**
- **Inhibition of Raf in a differentation system would result in exacerbation not reduction of tumors as has been observed**
- **The Raf-mediated upstream signalling activates the EGF-Receptor signalling in the presendce of drugs and resulted in application of a second drug against the EGF in Mal Mel Patients**
- **Mal Melanoma patients were treated with a Raf and a MEK inhibitor with predictable success**

References

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