Translational relevance of patient-derived colon tumor Xenografts (PDX) to correlate pathways abnormalities with response to anti-EGFR therapy

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Oncodesign

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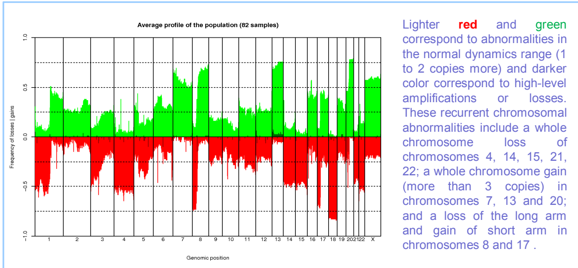
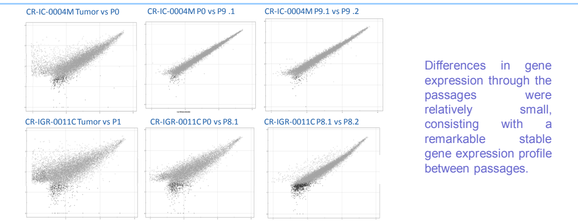
Frequence of abnormalities detected by CGH among all samples tested across the genome



Lighter red and green correspond to abnormalities in the normal dynamics range (1 to 2 copies more) and darker color correspond to high-level amplifications or losses. These recurrent chromosomal abnormalities include a whole chromosome loss of chromosomes 4, 14, 15, 21, 22; a whole chromosome gain (more than 3 copies) in chromosomes 7, 13 and 20; and a loss of the long arm and gain of short arm in chromosomes 8 and 17 .

Comparison of gene expression between primary tumor sample, early and late xenograft samples



Differences in gene expression through the passages were relatively small, consisting with a remarkable stable gene expression profile between passages.

Abstract

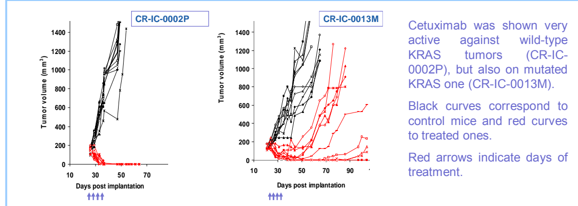
Patient-derived tumor Xenografts (PDXs) are considered to be more clinically-relevant preclinical models to evaluate the antitumor activity of therapeutic agents than the human tumor cell-derived Xenografts. Recently, through the CReMEC consortium, we established a collection of 52 colon PDXs, which were characterized in terms of whole genome gene expression, chromosomal abnormalities (CGH array), main gene mutations previously described in colon carcinomas, and response to cetuximab monotherapy (AACR 2010).

In the current study, we performed the comparative analysis of molecular profile with cetuximab response of each PDX in this preclinical setting.

Mutations were observed for KRAS in 21/52 PDXs (40%), for BRAF in 4/52 PDXs (8%) and for PIK3CA in 6/52 PDXs (12%). Seven models had 2 coexisting mutations. Gene expression comparison between KRASwt and KRASmt PDXs reveals a specific pattern of genes that could be associated to cetuximab resistance. Based on tumor growth inhibition ($\Delta T/\Delta C \leq 42$ %), the response to cetuximab was observed in 18/52 PDXs (35%), 30% (6/18) of them harboring KRAS mutation without other EGFR-Pathway abnormalities. Among the cetuximab-resistant PDXs, 64% (22/34) displayed KRAS, BRAF, and/or PIK3CA mutations. A positive association was established between the presence of theses mutations and the non-response to cetuximab therapy (p=0.02, Fisher's test). Finally, the survival of mice was evaluated, based on the time to reach a tumor volume of 750 mm3. Using logRank test, a significant survival advantage ($p<0.0001$) was observed for the cetuximab-treated KRASwt versus KRASmt PDXs. These results are in concordance with the ones previously reported in this clinical indication.

In conclusion, our results confirm the key role of KRAS mutation, but also of others abnormalities, as in EGFR-Pathway, in the cetuximab resistance. Nevertheless KRAS mutation is not always a driver for the absence of response to cetuximab. They also demonstrate that such a PDX collection could bring benefit for the evaluation of targeted therapies and to identify the molecular pathways involved in their sensitivity and innate resistance.

Cetuximab activity in patient-derived colon tumor Xenografts

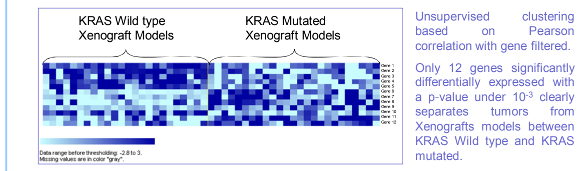


Cetuximab was shown very active against wild-type KRAS tumors (CR-IC-0002P), but also on mutated KRAS one (CR-IC-0013M).

Black curves correspond to control mice and red curves to treated ones.

Red arrows indicate days of treatment.

Class comparison analysis: *KRAS* mutated Xenografts models versus wild type.



Unsupervised clustering based on Pearson correlation with gene filtered.

Only 12 genes significantly differentially expressed with a p-value under 10^{-3} clearly separates tumors from Xenografts models between KRAS Wild type and KRAS mutated.

Material and methods

Molecular characterization

Molecular characterization

CGH array analysis: Evaluation of genome-wide, gene copy number was evaluated using a 244k CGH array Agilent technology.

Gene expression profiling: The analysis of gene expression was done using the U133A microarrays Affymetrix technology.

DNA sequencing: APC (exons 9 & 16), KRAS (exons 2 & 3), BRAF (exons 11 & 15), TP53 (exons 2 to 11), CTNNB1 (exon 3), PIK3CA (exons 10 to 21), FBXW7 (exons 3 to 10), EGFR (exons 18 to 21) and AKT1 (exon 4) were analyzed by Sanger direct.

Determination of Microsatellite Instability (MSI) status was determined as previously described(J. Natl Cancer Inst 2004, 96, 261-268).

In vivo pharmacological studies

Cetuximab (Imclone) at 12.5 mg/kg/adm, (Q3Dx2)x2 IP), mice bearing 100-200 mm^3 tumors at start of therapy (n = 8-10 per group).

Efficacy end Points: Tumor growth inhibition ($\Delta T/\Delta C$ value):

$$\Delta T/\Delta C\ (\%) = [(\text{median } T_{DayY} - \text{median } T_{DayX}) / (\text{median } C_{DayY} - \text{median } C_{DayX})] \times 100$$

(where DayY is the day of evaluation, and DayX is the day of initiation of therapy for treated [T] and control [C] tumor volumes).

Scoring criteria: - = (T/C) > 42 %; + = 10 < (T/C) ≦ 42 %; ++ = 0 ≦ (T/C) ≦10 % (stable disease); +++ = (T/C) <0 % (tumor regressions).

Statistical analysis

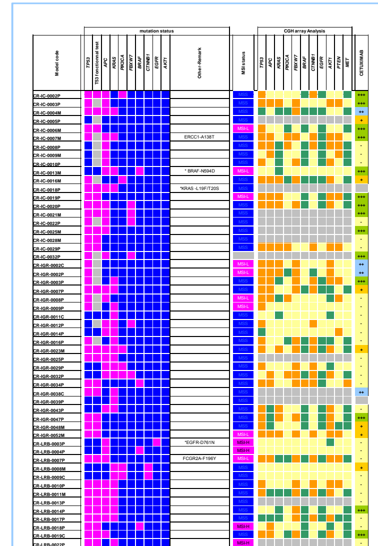
Pharmacology - Recursive partitioning method was performed using SAS JMP v9 software (16). The Fisher's test and all log rank analyses were performed using Everstat V5 (Sanofi based on SAS 8; SAS Institute Inc., Cary, NC). CGH - Acquired signals were normalized according to their dye and local GC% content using in-house scripts under the R statistical environment (http://cran.r-project.org). Resulting log2(ratio) were segmented using the CBS (13) algorithm implementation from the DNAcopy package for R. Aberration status calling was automatically performed for each profile according to its internal noise (variation of log2(ratio) values across consecutive probes on the genome). Gene Expression - into Resolver software (Rosetta Biosoftware, Kirkland, WA, USA) and BrB Array Tools.

Cetuximab sensibility and mutation profile of genes involved in EGFR/KRAS pathway

Cetuximab activity score		-	+	++	+++
MUTATIONS	*KRAS*	11	1	2	3
	KRAS / PI3KCA	3	2	0	0
	KRAS / BRAF	0	0	0	1
	BRAF	3	0	0	0
	PI3KCA	0	1	0	0
EGFR mutated pathway		17	4	2	4
EGFR wild-type pathway		10	3	2	10

The absence of response to cetuximab was significantly correlated with the mutational status taking altogether the mutations in BRAF, PIK3CA and KRAS genes (p=0.02, Fisher's test).

Summary of molecular and pharmacology analysis of 54 patients derived tumor models



Microsatellite analyses

- MSS Microsatellite stability
- MSI-L Low microsatellite instability
- MSI-LH Higth microsatellite instable

Hot spots analyses

- Mutation
- Wild-type

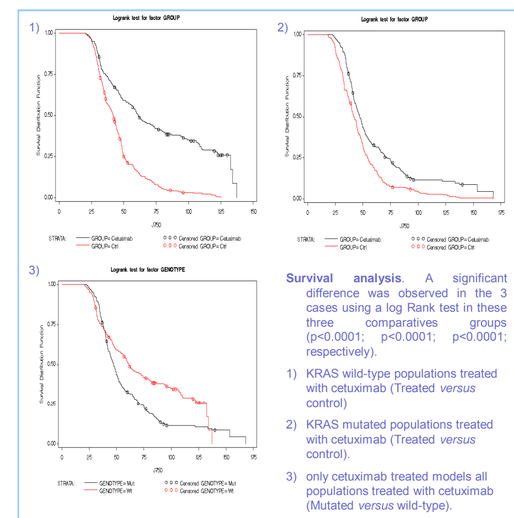
CNV (CGH) analyses

- No changes in genes copies
- Gain of 1 (or more) copies
- Loss of 1 (or less) copies

Cetuximab response scoring criteria

- \- = Δ(T/C) > 42 %;;
- \+ = 10 < Δ(T/C) ≦ 42 %;
- ++ = 0 ≦ Δ(T/C) ≦10 %;
- +++ = Δ(T/C) <0 %.

Survival analysis and the KRAS mutation status (Treated versus Control).



Survival analysis. A significant difference was observed in the 3 cases using a log Rank test in these three comparatives groups ($p<0.0001$; $p<0.0001$; $p<0.0001$ respectively).

1) KRAS wild-type populations treated with cetuximab (Treated *versus* control)
2) KRAS mutated populations treated with cetuximab (Treated *versus* control).
3) only cetuximab treated models all populations treated with cetuximab (Mutated *versus* wild-type).

Conclusion

The comparison of the survival curves of cetuximab-treated mice xenografted with wild type KRAS and mutant KRAS tumors shown significant difference as observed in human clinical trial.

We confirm the key role of KRAS EGFR/KRAS pathway mutation in the cetuximab resistance.

Gene expression comparison reveals a specific pattern of 12 genes that could be associated to cetuximab response.

PDX collection could bring benefit for the evaluation of targeted therapies and to identify the molecular pathways involved in their sensitivity and innate resistance.