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BRIEF REPORT



When monoclonal antibodies are not monospecific: Hybridomas frequently express additional functional variable regions

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ABSTRACT

Monoclonal antibodies are commonly assumed to be monospecific, but anecdotal studies have reported genetic diversity in antibody heavy chain and light chain genes found within individual hybridomas. As the prevalence of such diversity has never been explored, we analyzed 185 random hybridomas, in a large multicenter dataset. The hybridomas analyzed were not biased towards those with cloning difficulties or known to have additional chains. Of the hybridomas we evaluated, 126 (68.1%) contained no additional productive chains, while the remaining 59 (31.9%) contained one or more additional productive heavy or light chains. The expression of additional chains degraded properties of the antibodies, including specificity, binding signal and/or signal-to-noise ratio, as determined by enzyme-linked immunosorbent assay and immunohistochemistry. The most abundant mRNA transcripts found in a hybridoma cell line did not necessarily encode the antibody chains providing the correct specificity. Consequently, when cloning antibody genes, functional validation of all possible VH and VL combinations is required to identify those with the highest affinity and lowest cross-reactivity. These findings, reflecting the current state of hybridomas used in research, reiterate the importance of using sequence-defined recombinant antibodies for research or diagnostic use.

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Introduction

Most scientists using monoclonal antibodies are unaware that a considerable number exhibit off-target binding, despite recently increased awareness of general problems with antibody specificity.^{1–9} Disturbingly, the degree of monoclonal antibody off-target binding can vary with different antibody lots, resulting in results that have affected patient stratification and treatment.^{10–13} While off-target binding may be due to cross-reactive recognition of epitopes similar to those in the intended target (mimotopes), it can also arise from the co-expression of additional productive, but non-target-binding, antibody chains, which arise from the way hybridomas are generated. Hybridomas are *in-vitro* generated,

tetraploid, cancer-derived, artificial cells. The number of chromosomes in hybridomas,^{14–17} and fusion partners,^{18,19} vary widely, with individual hybridomas developing up to 50 additional chromosomes under some growth conditions.¹⁴ Early fusion partners secreted an additional light chain, which has been eliminated in most clones derived from the original MOPC21 clone (Sp2/0, NS0, OUR-1 and P3-X63-Ag8.653).²⁰ Not eliminated is a frequently transcribed unproductive (aberrant) κ chain,^{21,22} which can complicate antibody gene cloning efforts if not removed.^{23–25} Although literature reports on the presence of additional productive antibody chain mRNAs in hybridomas have accumulated,^{20,23,26–33} how frequently these are produced is not clear. As

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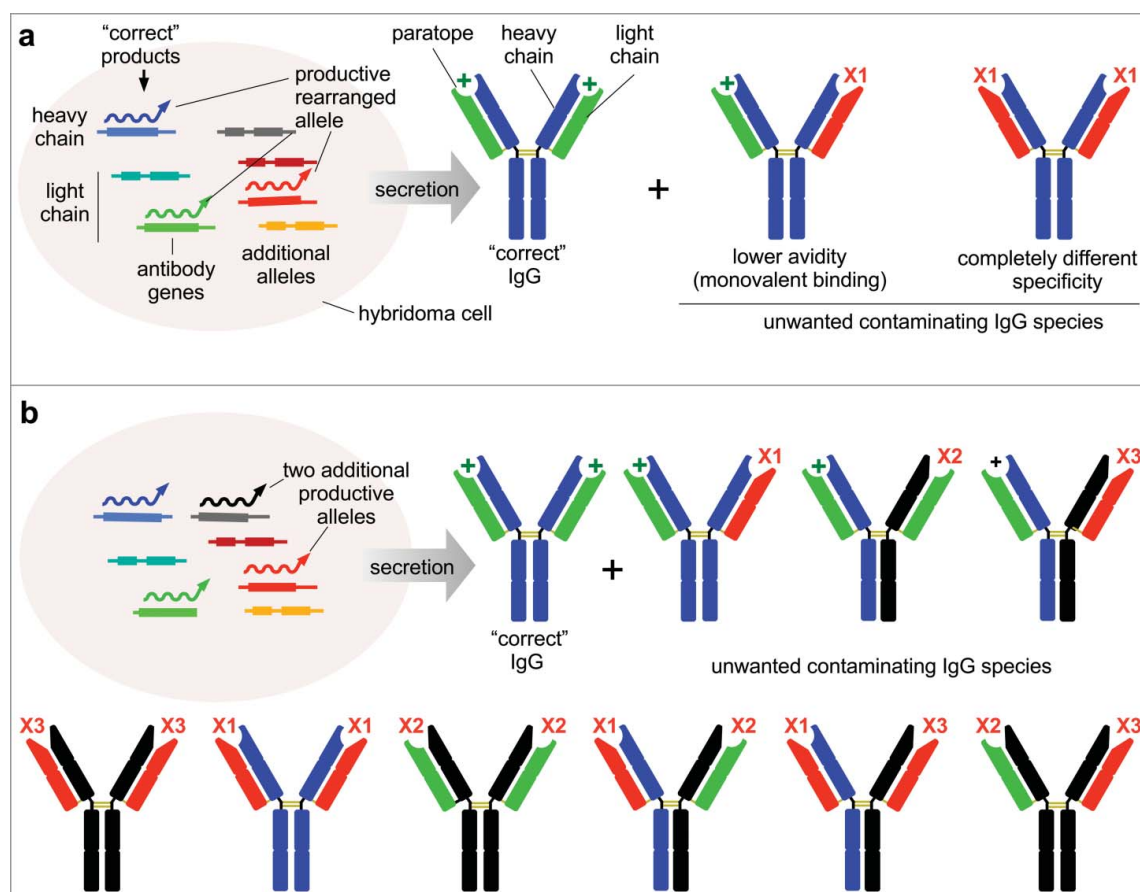


Figure 1. Additional heavy or light chains secreted by a hybridoma create additional binding sites in a combinatorial way. **a**, hybridomas with one additional chain (example given here: LC) generate three different IgGs with two different antigen binding sites, while **b**, ten different IgGs with combinations of four different paratope structures are produced if both one additional LC and HC are present. + correct paratope, X1-X3 additional paratopes.

the combination of heavy chain (HC) with light chain (LC) in the secretory pathway is largely random, a single additional LC will result in three different IgG species (Fig. 1A), while two HCs and two LCs will produce 10 different IgG species with four structurally distinct antigen binding sites (Fig. 1B).³⁴

The possible reasons why hybridomas may express more than one HC or LC are manifold and illustrated in Fig. 2. They include mixed colonies in which hybridomas have not been cloned to purity, the simultaneous fusion of myeloma partners to more than one spleen cell,³⁵ or to a spleen cell with two productive rearrangements, post fusion rearrangements or mutations, particularly after prolonged *in vitro* cultivation,²⁸ and all combinations thereof. Such additional chains can cause problems in both research and diagnostic applications,^{10,36,37} particularly when it is assumed that a monoclonal antibody is a defined monospecific molecular entity.

This study provides the first systematic analysis of the problem, following recent proposals to improve the quality of research antibodies by expressing them recombinantly and defining them by their sequences.^{2,3}

Results

A significant fraction of hybridomas produces additional chains

While the expression of the non-productive common aberrant light chain²³ is well documented, there are numerous

published examples of other antibody HC and LC mRNA heterogeneities found in hybridomas^{20,23,26-29} that can cause problems when trying to clone functional VH/VL combinations. However, because these studies were all focussed on obtaining one specific correct recombinant antibody, they provide no information on the overall prevalence of additional productive antibody chains, and their general impact on mAb specificity and functionality. To understand the extent of the problem, we assembled a large multicentric dataset from the hybridoma cloning community. Data generated by seven laboratories over the past twenty years were assembled, and a total of 185 hybridomas were included in the analysis (Supplementary Table 1). Most of the monoclonal antibodies produced by these hybridomas are commercial products. Unlike previous studies that reported additional productive antibody chains,^{20,23,26-33} the hybridomas we analyzed were not in any way preselected for cloning difficulties, but sequenced because there was a desire to either produce the antibodies as recombinants, or have access to their genes. Our unbiased hybridoma collection is diverse, but representative of antibodies generally used in the scientific community. Sequences were obtained using a number of different standard methods, including RACE,²⁶ V region PCR²⁷ and next-generation sequencing (NGS). In some cases, efforts to eliminate the known unproductive aberrant light chain were taken.^{20,23,38} Correct VH/VL pairs were identified for all 185 hybridomas analyzed. Of these,

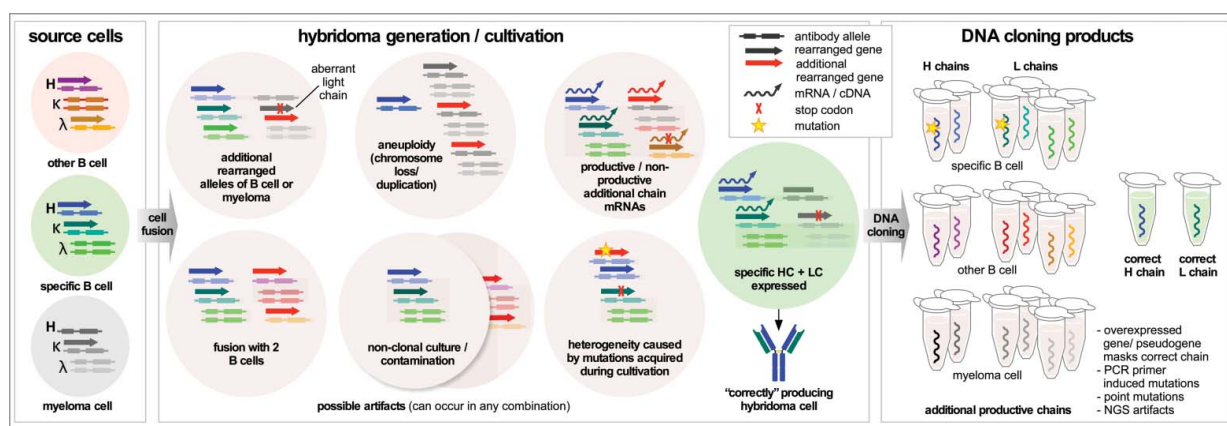


Figure 2. Additional immunoglobulin DNA isolated from hybridomas can originate from many different sources. Note that all identified factors can also be present simultaneously.

126 (68.1%) were found to contain only one productive VH gene and one productive VL gene, while 59 (31.9%) expressed one or more additional productive V gene. Among the latter, 53 (28.6% of all analyzed hybridomas) contained an additional productive VL, 2 (1.1%) had an additional productive VH, and 4 (2.2%) had at least one additional VH in addition to additional VL (Table 1, and Supplementary Table 1), expected to result in non-mono-specific IgG mixtures, as depicted in Fig. 1b.

Additional productive antibody chains can impair affinity and specificity of IgG purified from hybridoma supernatants

In the dataset described above, the presence of an open reading frame was used to identify productive chains. IgGs with a mixture of chains are expected to have impaired functionality in two ways: 1) the number of “correct” binding sites present per unit of protein will be reduced, resulting in lower apparent binding activity; and 2) the presence of more than one paratope structure (Fig. 1) can lead to additional off-target reactivities, thereby compromising specificity. To explore the magnitude of these effects, enzyme-linked immunosorbent assay (ELISA) was used to compare binding of affinity-purified hybridoma IgG to corresponding recombinant IgG produced from all possible VH/VL combinations of a randomly selected subset of hybridomas (Table 2). Six of seven tested hybridomas in this dataset contained additional productive sequences. In one case (pol2ser5), the most abundantly expressed HC/LC sequences (recAb VH1/VL1) did not provide the expected specificity, and specific binding to the correct target was

found when a less abundant VL (VL2) was paired with VH1. In another case (SUZ12), IgG constructed by pairing the secondary VH and VL (VH2/VL2) created strong off-target binding, even within the small number of antigens tested. In one case the “correct” HC/LC combination (pol2) also showed polyreactivity. Interestingly, all seven antibodies purified after recombinant expression, including EED, where only a single VH and VL were identified, showed both stronger reactivity per unit protein and higher relative specificity compared to the IgG purified from the corresponding hybridoma supernatants.

In a second set of experiments, we carried out immunohistochemistry staining using monoclonal antibodies purified from six randomly chosen different hybridomas or their corresponding recombinant antibody versions. Both hybridoma and recombinant antibodies were purified on Protein A (IgG2a and IgG2b) or Protein G (IgG1 and IgG3). The purified hybridoma antibodies were diluted to the point that they were weakly positive for staining (three of the antibodies were additionally tested at higher concentrations). Recombinant versions of the hybridoma antibodies were diluted to the same concentration, and sequential sections of human tissues were fixed in 10% formalin and stained with either hybridoma or recombinant antibody.

As shown in Fig. 3, purified antibodies from recombinant expression showed higher sensitivity than antibodies purified from hybridoma supernatants when additional productive VH or VL chains are identified (Fig. 3A: cytokeratin 7, β 2 microglobulin, Fig. 3B: calponin, EpCAM). Interestingly, this was also observed for the examples in which only a single productive VH and VL gene pair was identified in the hybridoma (Fig. 3C: MUC1, WT1), mirroring the ELISA results obtained with the anti-EED antibody described above. The molecular mechanism responsible for this phenomenon, which so far was observed in two different hybridomas in two different assays, remains to be identified. In addition to the improved sensitivity, some of the recombinant antibodies in which additional chains were found in the hybridoma (cytokeratin 7, EpCAM) also showed distinct off-target binding, while the β 2 microglobulin antibody, where two additional productive chains were found, showed

Table 1. Hybridomas commonly carry additional productive antibody chains. The analysis of 185 different hybridomas for the presence or absence of additional productive variable regions is shown.

Class	Numbers	Percentage
Correct VH/VL, no additional chains	126/185	68.1
Additional productive VL	53/185	28.6
Additional productive VH	2/185	1.1
Additional productive VL+VH	4/185	2.2

Table 2. Binding reactivities of IgG purified from hybridoma supernatants compared to different recombinant light and heavy chain combinations. Combinations of major HC and LC sequences found after NGS of 5' RACE products from seven hybridomas were produced and a chessboard ELISA binding assay on antigens was done (signals given in standardized arbitrary units).

Target name	Active Motif prod no	Purified IgG	EST CONC (ng/mL)	EED	EZH2	Hh3	Hh3.3	P300	POL2 p0	POL2 pS2	POL2 pS5
EED	61203.0	Hybridoma1	1000.0	146.6	258.8	0.7	0.7	0.8	0.6	0.8	0.8
		Hybridoma1	100.0	9.0	31.3	0.6	0.6	0.7	0.5	0.7	0.6
		VH1 VL1	20.0	104.9	126.0	1.4	1.4	0.9	0.9	0.7	0.8
EZH2	39875.0	Hybridoma2	1000.0	0.2	301.6	4.1	1.2	1.0	0.5	0.5	0.5
		Hybridoma2	100.0	0.4	35.4	0.7	0.7	0.9	0.6	0.7	0.7
		VH1 VL1	20.0	0.3	432.4	9.3	4.2	0.5	0.5	0.5	0.5
		VH2 VL1	20.0	0.5	1.2	1.6	2.1	0.5	0.5	0.5	0.5
SUZ12	39877.0	Hybridoma3	1000.0	0.7	337.9	1.8	6.0	1.8	1.3	1.5	1.7
		Hybridoma3	100.0	0.6	32.7	1.0	1.0	1.6	1.0	1.2	1.3
		VH1 VL1	20.0	0.4	82.2	1.2	1.3	0.7	0.6	0.6	0.6
		VH2 VL2	20.0	0.3	8.6	1.6	2.0	73.4	3.9	105.2	274.5
		VH1 VL2	20.0	0.4	1.7	1.3	1.5	0.8	0.6	1.5	2.1
		VH1 VL3	20.0	0.4	0.9	1.5	1.3	0.7	0.7	0.6	0.7
		VH2 VL3	20.0	0.3	0.7	0.9	1.0	0.5	0.5	0.6	0.8
		VH2 VL1	20.0	0.3	0.9	3.1	0.9	0.5	0.7	0.5	1.1
H3total	61475.0	Hybridoma4	900.0	0.6	18.6	60.0	53.5	1.4	1.3	1.3	1.4
		Hybridoma4	90.0	0.6	16.9	2.0	1.8	1.8	1.2	1.3	1.4
		VH1 VL1	20.0	0.6	2.0	58.8	42.2	1.2	1.4	1.3	1.3
		VH1 VL2	20.0	0.5	1.3	2.1	1.7	0.9	1.1	1.0	1.0
pol2	39097.0	Hybridoma6	200.0	2.3	25.1	0.9	1.0	3.9	1.1	15.8	44.9
		Hybridoma6	20.0	0.5	18.5	0.8	0.8	1.1	0.8	1.1	1.4
		VH1 VL1	20.0	0.6	8.5	2.3	3.3	55.1	9.8	71.9	274.8
		VH2 VL1	20.0	0.3	0.9	1.3	1.6	1.4	0.6	0.7	3.2
pol2ser2	61083.0	Hybridoma7	1000.0	0.4	17.9	0.7	0.7	1.0	0.6	29.6	0.8
		Hybridoma7	100.0	0.4	16.1	0.6	0.7	1.3	0.7	0.9	0.8
		VH1 VL1	20.0	0.2	1.3	1.1	1.2	0.7	0.7	424.9	1.5
		VH1 VL2	20.0	0.3	0.7	0.9	1.1	0.6	0.4	0.5	0.6
		VH1 VL3	20.0	0.2	1.3	1.0	1.2	0.4	0.4	0.5	0.5
pol2ser5	61085.0	Hybridoma8	1000.0	0.4	15.8	0.8	0.9	1.0	1.4	21.8	11.7
		Hybridoma8	100.0	0.3	17.2	0.8	0.8	1.2	0.8	1.2	1.0
		VH1 VL1	20.0	0.2	0.7	1.3	1.2	1.2	1.9	288.1	261.3
		VH1 VL2	20.0	0.3	1.2	1.5	1.5	0.8	0.6	1.8	447.5

Pink boxes indicate the expected reactivity. In each case, VH1 and VL1 were the most abundant chains identified by NGS. EED: Human embryonic ectoderm development protein; EZH2 complex: Enhancer of Zeste homolog 2 (EZH2) complex contains EZH2, EED and Suz12 proteins. H3: Histone H3; pol2: RNA polymerase II; pSer5 and pSer2 are antibodies that recognize phosphorylated epitopes in RNA polymerase II.

no apparent off-target binding at least in the particular tissues used for the experiments. There was no obvious correlation between the mRNA level of additional productive V gene transcripts, with some occurring at <0.1% of total antibody transcripts, and the degree of off-target binding, reflecting the inability to predict binding by off-target paratopes generated by additional chain combinations for any individual tissue or sample.

Discussion

Additional sequences have different origins

Within the set of 185 hybridoma clones analyzed here, we were able to find all previously reported genetic changes and aberrations, including both productive and unproductive HCs and/or LCs originating from different V regions, possible recombination intermediates, small indels,

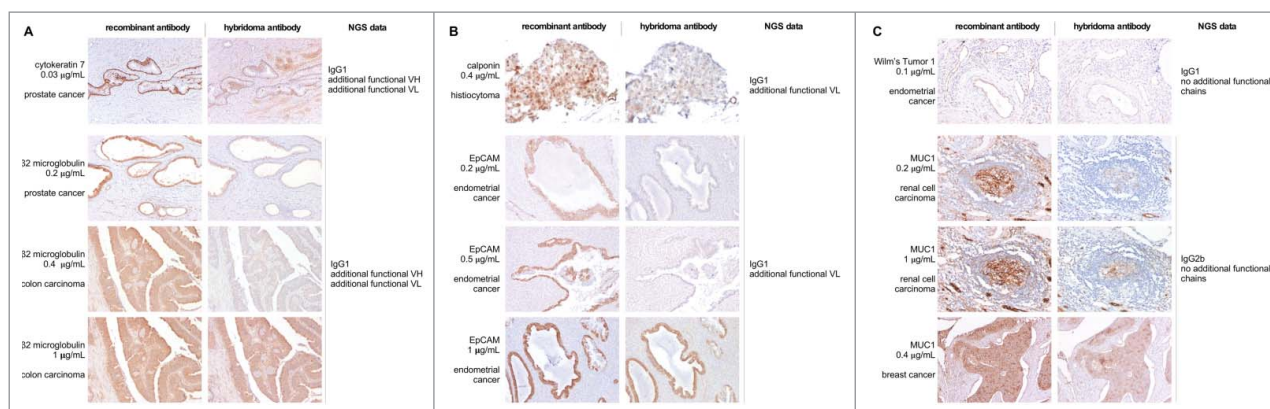


Figure 3. Recombinant antibodies show greater specificity and sensitivity in immunohistochemistry. Hybridomas expressing antibodies with more than one additional functional chain (3A), an additional functional VL (3B), and antibodies with no additional functional chains (3C) were identified by next-generation sequencing. Antibodies purified from either hybridoma supernatant or after recombinant antibody expression in HEK cells, were tested on adjacent sections at identical concentrations. For each antibody, the lowest concentration of purified hybridoma antibody that provided a signal was used, as well as higher concentrations for β 2 microglobulin, EpCAM and MUC1. Recombinant antibodies show stronger and more specific binding compared to the hybridoma antibodies. All micrographs were taken with identical photographic parameters, with the adjustment of white points to identical levels being the only correction made.

frameshifts, point mutations and aberrant light chains, as well as combinations thereof. The many different potential origins for these additional chains are illustrated in Fig. 2. Significantly, many additional sequences could not be attributed to the fusion partner. Of the 185 hybridomas analyzed over this 20-year period, about half produce additional mRNAs and about one-third produce at least one additional productive chain. Rat hybridomas showed a much higher prevalence of additional productive chains (78.3%; 29/37) than murine hybridomas (19.9%; 29/146). Five of six of the hybridomas with additional productive additional HCs were of mouse origin. Both sequencing methods identified a similar fraction of hybridomas with additional productive chains: by NGS sequencing, 45 of 136 analyzed clones (33.1%) were found to express at least one additional productive chain, while PCR cloning identified 14 of 49 analyzed clones (28.5%) with at least one additional productive chain. In addition to the expected aberrant LC and V regions originating from the myeloma fusion partner, sequences derived from completely unrelated V genes were not uncommon for both LC- and HC-encoding mRNAs, and these could be both productive and unproductive. The sequences may be derived from the combination of fusion partners with multiple spleen cells, or spleen cells that have rearranged allelically excluded chromosomes, so-called “allelic inclusion”³⁹ found in 0.4% of human B cells. It is also possible that alleles not used to produce the specific chains are somehow re-activated. Hybridoma cells originally contain at least four HC and eight LC alleles (Fig. 2), and molecular regulation mechanisms active during B cell development cannot be expected to be as tight in a tetraploid/aneuploid cancer/B-cell fusion. B cells can carry out additional recombination during development, and while these steps are usually complete before class switching, some components may be re-activated in hybridomas, as clearly demonstrated by the observation of spontaneous class-switching in rat and mouse hybridomas.⁴⁰

From the beginning, researchers were aware of, and tried to mitigate, the problem of additional chains encoded by

fusion partners. Köhler and Milstein’s original publication used the cell line P3/X63Ag8, which secreted the myeloma protein P3 (an IgG1/ κ). Subsequently NS1,⁴¹(for immunoglobulin non-secretor clone number 1), an antibody HC loss cell line, and NS0⁴²(a spontaneous LC loss variant of NS1) were derived. NS0 expressed a LC mRNA with a characteristic frameshift mutation (Clark M., unpublished observations) common to other cell lines derived from MOPC21.²¹ Similarly, Sp2/0,⁴³ an immunoglobulin non-producing hybridoma cell line, was derived from Sp2, an anti-SRBC antibody made by fusion with P3/X63Ag8.³³ Using Köhler and Milstein’s terminology, the original Sp2 made four chains termed HLGK (H and L being spleen cell derived and G and K being myeloma derived). Through a process of subcloning and selection, they sequentially isolated clones expressing HLK, HL, L and finally eliminated all antibody expression in Sp2/0.⁴³ A third chain loss cell line, termed P3/X63Ag8.653,⁴⁴ was also derived directly from P3/X63Ag8. The sequences of these fusion partner V regions can be found on: imgt.org/IMGTeducation/IMGTlexique/U/Unproductivetranscript.html.

Identifying the genes encoding functional antibodies

The best way to identify the correct sequence combination of heavy and light chains is to use the recombinant version of the antibody alongside the antibody made in a hybridoma in the same functional binding assay (e.g., see Table 2 and Fig. 3). This functional binding assay of the reassembled recombinant antibody should demonstrate the same, or better, affinity and specificity as the IgG derived from hybridoma supernatant, particularly if additional VH/VL cDNAs were detected. All pairings of the individual VL and VH DNAs found by NGS or all PCR products amplified with different primer sets should be combined and tested individually (Table 2). Transient expression systems, usually based on HEK293 cells, are now available to produce sufficient material for functional tests from small-scale transient cultivation.⁴⁵ Special attention must be taken to control for specificity, as several LCs may provide similar affinities in combination with the same HC, while the correct pair

provides the best specificity, as illustrated, for example, by pol2ser5 in Table 2.

In these ELISA experiments, we compared monoclonal antibodies to recombinant antibodies. Since monoclonal antibodies are very rarely affinity purified on their antigen (a procedure so far assumed to be unnecessary), additional IgGs with no target binding activity (Fig. 1) will contaminate the IgG fraction from all hybridomas in which additional productive chains are expressed. First, this dilutes the “correct” antibody and leads to lower signals per purified total IgG protein, an effect clearly evident for the clones tested in this study. Second, the additional uncharacterized specificities may lead to unexpected results if applied to experimental setups and assays different from those used for the original hybridoma characterization. The presence of additional chains can lead to off-target binding, but this may not always be the case, as the epitope recognized by the additional paratope needs to be present in the particular sample and may be affected by preparation, fixation or incubation conditions.

An alternative to identifying the correct sequence combination of heavy and light chains is the generation of small phage display libraries from which functionally correct chain pairs can be selected by antigen binding.^{27,28} This approach has the caveat that, in contrast to human sequences,⁴⁶ some rodent antibodies are produced inefficiently in the scFv or Fab format typically used in *E. coli*.

In conclusion, although there can be significant inherent diversity within antibody DNA derived from a hybridoma, the methods available to unambiguously identify the correct sequences are robust, available and well proven. Given the improved sensitivity and specificity of recombinant antibodies, and the unlimited reproducibility that arises from knowledge of the antibody sequence, and long-term stability of transfected cell lines compared to hybridomas, the observations described here reiterate the importance of transitioning to the wholesale use of recombinant production of sequence-defined antibodies.^{2,3,47}

Materials and methods

A total of 116 laboratories assumed to be involved in hybridoma cloning work were contacted to determine whether they could contribute suitable datasets, and 15 positive responses were received. Sequence sets of 7 labs finally met the inclusion criteria, which, in addition to functional clones, required that a sequence set cover all hybridomas analyzed in that lab within a given timeframe without any preselection for “problematic” cases or cloning problems. All hybridomas studied were sequenced because of the need to either produce the antibodies as recombinants, or to gain access to their genes. Data for ~90% of the analyzed hybridomas were contributed by three commercial antibody companies, Miltenyi Biotec, Absolute Antibody and NeoBiotechnologies, with roughly 1/3 of the sequences coming from each company. Smaller numbers (≤ 6 per set) were reported by the International Centre for Genetic Engineering and Biotechnology (Burrone lab), University of Heidelberg (Dübel lab), A*Star p53 laboratory (Lane lab) and University of Freiburg (Wolf lab).

Sequences were obtained over a 20-year period using using a number of different standard methods, including RACE²⁶ or V

region PCR²⁷ (49 hybridomas, Miltenyi and academic labs), while the majority (136 hybridomas) were analyzed by NGS within the past 3 years (Absolute Antibody and NeoBiotechnologies). In some cases, PCR-based methods included published measures to eliminate known unproductive aberrant light chains (A*Star).^{20,23,38} which are not expected to have any major effect on the detection of other chains than the known aberrant light chain.

For the antibodies tested by ELISA (Table 2), NGS forward and reverse sequence reads were assembled, clustered, and quantitated by number of sequence counts in each cluster. Clusters with >5 reads were then aligned and manually annotated. The HC and LC sequence with the most counts was deemed the primary sequence (for heavy and light chain). Additional sequences were either productive minor variants of the primary sequence with point mutations or small indels, non-productive sequences (premature stop codon), or sequences clearly derived from an entirely different V gene when compared to the primary sequence. Each mutant sequence had to be supported by at least 5 independent sequence reads to be included in the table, to exclude sequencing artifacts. All productive pairs were tested.

For the six antibodies tested by immunohistochemistry (Fig. 3), the entire transcriptome was sequenced by NGS to eliminate any primer bias, and all possible immunoglobulin sequences were identified; where additional productive VH or VL sequences were found they are indicated in Fig. 3. VH and VL chains were cloned and expressed in mammalian expression vectors as full-length antibodies in transient transfections. For each individual hybridoma line, every possible VH and VL combination was recombinantly expressed to produce a full-length IgG antibody. Heavy and light chain expression vectors were mixed in equal molar ratio and transfected. After 48 hours, antibody containing supernatants were harvested.




For immunohistochemistry, hybridoma and recombinant antibodies were purified on Protein A (IgG2a and IgG2b) or Protein G (IgG1 and IgG3). Five micron thick sections were cut from formalin-fixed, paraffin-embedded human tumor specimens and mounted on charged slides (Mercedes Medical), deparaffinized in SlideBrite, washed with 100% ethanol, followed by rehydration in graded ethanol (95%, 70% and 50%). Endogenous peroxidase was blocked by immersing slides in 3% hydrogen peroxide for 5 minutes followed by washing in distilled water. Antigen retrieval was performed in 10 mM citrate buffer (pH 6.0) by heating slides in a pressure cooker (Decloaking Chamber, Biocare Medical) at 125°C for 20 minutes. Tissue slides were cooled to 80°C and washed in water. All primary antibodies were diluted in 10 mM phosphate-buffered saline (PBS), pH 7.4, containing 3% bovine serum albumin and 0.05% azide. The antibody concentrations used are given in Fig. 3. After 30 minutes incubation with antibodies, slides were rinsed and binding was revealed with goat anti-mouse horseradish peroxidase polymer detection (PIR080, ScyTek Laboratories) applied to tissue sections for 15 minutes at room temperature and rinsed in PBS as before. Sections were finally incubated in 3,3'-diaminobenzidine (Scytek Laboratories) for 5 minutes. Counterstaining was done with hematoxylin. Negative controls consisting of diluent with no antibody were used in all experiments.

The ELISA reactivity of the sequence-defined recombinant antibodies (in different combinations) was compared to their corresponding hybridoma parental antibodies. The binding of 18 recombinant antibodies plus the 7 parental hybridoma antibodies were assayed for binding to 8 different protein antigens (EED: LifeTech #11307-H20B; EzH2 complex: Active Motif #31337; Histone H3: AM #31271; H3.3: AM #31295; P300: AM #31124; RNA Polymerase II by ELISA; Pol2 p0 peptide AbCam #17564/; Pol2 pS2 peptide: AbCam #12793; Pol2 pS5 peptide: AbCam 18488) by ELISA using standard protocols by adding antibody supernatant to the antigen-coated plates at the concentrations indicated in Table 2. ELISAs were also used to confirm expression and provide a rough quantification of the amount of full-length IgG antibody in the supernatant.

Disclosure statement

ARMB, NDT, HT, ICW, AKT, SA, CLB, BJ, SFA, FF, AF, DLP, MC and SD are shareholders, advisors or employees of companies involved in the production of recombinant antibodies.

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