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Cancer/Testis (CT) antigens - a new link between gametogenesis and cancer

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It is a little acknowledged fact that the discipline of tumor immunology has been the source of many findings of critical importance in cancer-related as well as cancer unrelated fields. For example, it was the search for tumor antigens that led to the discovery of the CD8 T cell antigen (1) and the concept of differentiation antigens (2) (and the CD system for classifying cell surface antigens), and to the discovery of p53 (3). The immunogenetic analysis of resistance to viral leukemogenesis provided the first link between the MHC and disease susceptibility (4), and interest in the basis for non-specific immunity to cancer gave rise to the discovery of TNF (5). These are only a few examples from our own work, and I could site many other key insights into important biological issues that have come from other groups whose prime motivation was understanding the role of the immune system in cancer. The reason for this historical prelude is to give some perspective to another area of tumor immunology that I believe holds great promise for a deeper understanding of cancer, and this has to do with the category of antigens referred to as cancer/testis (CT) antigens, first recognized as targets for CD8 T cell recognition of autologous human melanoma cells (6, 7). The molecular definition of these antigens was a culmination of prior efforts to establish systems and methodologies for the unambiguous analysis of humoral (8) and cellular (9) immune reactions of patients to autologous tumor cells (autologous typing), and this approach of autologous typing also led to the development of SEREX (serological analysis of cDNA expression libraries) for defining the molecular structure of tumor antigens eliciting a humoral immune response (10). As a consequence of T cell epitope cloning and SEREX analysis, a growing number of CT antigens have now been defined, and other approaches, e.g., representational difference analysis and in silico surveys of cDNA data banks are adding to the list (Table 1 and references therein).

There are now 14 genes or gene families that code for products with the following characteristics: (i) mRNA expression in normal tissues is restricted to testis, fetal ovary, and placenta, with little or no expression detected in adult ovary. (ii) mRNA expression in cancers of diverse origin is common - up to 30 - 40% of a number of different cancer types, e.g., melanoma, bladder cancer, sarcoma express one or more CT antigens. (iii) The X chromosome codes for the majority of CT antigens, but a number of more recently defined CT coding genes have a non-X chromosomal locus. (iv) In normal adult testis, expression of CT antigens is primarily restricted to immature germ cells -, e.g., spermatogonia (31). However, a recently defined CT antigen, OY-TES-1, is clearly involved in late stages of sperm maturation (see below). In fetal ovary, immature germ cells (oogonia/primary oocytes) express CT antigens, whereas oocytes in the resting primordial follicles do not (32). In fetal placenta, both cytotrophoblast and syncytiotrophoblast express CT antigens, but in term placenta, CT antigen expression is weak or absent (33). (v) A highly variable pattern of CT antigen expression is found in different cancers, from tumors showing only single positive cells or small cluster of positive cells to other tumors with a generally homogeneous expression pattern (31, 34). (vi) The function of most CT antigens is unknown, although some role in regulating gene expression appears likely. Two CT antigens, however, have known roles in gamete development - SCP-1, the synaptonemal complex protein, is involved in chromosomal reduction during meiosis (35), and OY-TES-1 is a proacrosin binding protein sp32 precursor thought to be involved in packaging acrosin in the acrosome in the sperm head (36). (vii) There is increasing evidence that CT expression is correlated with tumor progression and with tumors of higher malignant potential. For instance, a higher frequency of MAGE mRNA expression is found in metastatic vs. primary melanoma (37) and in invasive vs. superficial bladder cancer (38), and NY-ESO-1 expression in bladder cancer is correlated with high nuclear grade (39). (viii) There appears to be considerable variation in the inherent immunogenicity of different CT antigens as indicated by

specific CD8+ T cell and antibody responses in patients with antigen positive tumors. To date, NY-ESO-1 appears to have the strongest spontaneous immunogenicity of any of the CT antigens - e, g, up to 50% of patients with advanced NY-ESO-1+ tumors develop humoral and cellular immunity to NY-ESO-1 (40, 41).

Table 1. Human CT (Cancer/Testis) Antigens.

CT[a]	System	# Genes	Chromosome Location	Detection System[b]	References
1	MAGE	16	Xq28/Xp21	T, Ab	7,10,12,13
2	BAGE	2	Unknown	T	14
3	GAGE	9	Xp11	T	15,16
4	SSX	>5	Xp11	Ab	10,17
5	NY-ESO-1 LAGE-1	2	Xq28	Ab, T, RDA	18,19
6	SCP-1	3	1p12-p13	Ab	20
7	CT7 / MAGE-C1	1	Xq26	Ab, RDA	21,22
8	CT8	1	Unknown	Ab	23
9	CT9	1	1p	Ab	24
10	CT10 / MAGE-C2	1	Xq27	RDA, Ab	25,26
11	CTp11	1	Xq26 -Xq27	-c	27
12	SAGE	1	Xq28	RDA	28
13	cTAGE-1	1	18p11	Ab	29
14	OY-TES-1	2	12p12-p13	Ab	30

[a]Numbered according to the CT nomenclature proposed by Old & Chen (11).

[b]Abbreviations: Ab, antibody; T, CD8+ T cell; RDA, representational difference analysis.

[c]Defined by differential mRNA expression in a parental vs. metastatic melanoma cell variant.

As expected, most attention has been given to the potential of CT antigens as targets for cancer vaccine development, and, other than mutational antigens and virus encoded antigens, they clearly represent the most specific tumor antigens discovered to date. However, I believe they also provide a new way to think about cancer and its evolution during the course of the disease. The starting point for this view is the fact that CT antigen expression is restricted to early germ cell development and cancer. Germ cells give rise to gametes (oocytes and spermatocytes) and trophoblastic cells that contribute to the formation of the chorion and the placenta. Primitive germ cells arise in the wall of the yolk sack and during embryogenesis migrate to the future site of the gonads. In oogenesis, the process begins before birth, with oogonia differentiating into primary oocytes. The primary oocytes, which reach their maximal numbers during fetal development, are arrested at the initial phase of meiosis, and do not renew and complete meiosis until ovulation and fertilization. In contrast, spermatogenesis begins at puberty and is a continuous process of mitosis to maintain the spermatogonia pool and meiosis to

generate the mature sperm population. CT antigens, like SCP-1 and OY-TES-1, the proacrosomal binding protein precursor, are clearly important in gametogenesis, and it is likely that the other CT antigens with their restricted expression in gametes and trophoblasts also play a critical role in early germ cell development.

What then causes the reactivation of CT genes during cancer development and progression? And does the expression of these genes contribute to the malignant behavior of cancer cells?

One possibility to account for aberrant CT expression in cancer relates to the global demethylation associated with certain cancers (42). The promoter region of the MAGE gene has binding sites for transcriptional activators and these are methylated in normal somatic cells but demethylated in MAGE-expressing cancer cells and testis. Although cancer-associated demethylation could therefore account for CT (MAGE) expression in tumors, it does not easily accommodate the usual observation of non-coordinate expression patterns (sets) of different CT antigens in most tumors. Also, the marked heterogeneity in CT expression in some tumors (34, 43) is also not easily explicable by a global demethylation process. Another mechanism for reactivating CT expression in cancer has to do with mutations in regulatory regions of the CT genes. Although no mutations in CT genes have been found to date, more extensive sequencing, particularly in the promoter region, needs to be done before this can be excluded. However, mutation of CT genes is unlikely to be a common mechanism for the induction of CT expression in cancer. Another possibility to account for the appearance of CT antigens in cancer is the induction or activation of a gametogenic program in cancer. According to this view, the different CT sets seen in cancer would replicate the corresponding sets of CT antigens normally expressed during different stages of gametogenesis or trophoblast development. What would activate this specific gene program in cancer? Triggering events for inducing the gametogenic program could be a mutation in an as yet unidentified master switch in germ cell development, or an activation of this master switch by threshold mutations in oncogenes, suppressor genes, or other genes in cancer. It is also possible that activation of a single CT gene could be the switch for activating other genes in the gametogenic program. Supporting evidence for this idea comes from the study of synovial sarcoma, where a translocation event involving the SYT gene on chromosome 18 and the SSX-1 or SSX-2 gene on chromosome X is associated with high expression of unrelated CT antigens, such as NY-ESO-1 and MAGE (44, 45). Extending this line of reasoning and relating it to the role of demethylation in the appearance of CT antigens, a demethylation state in cancer (whatever its cause) could induce the gametogenic program and result in the activation of silent CT genes. Alternatively, demethylation may be an intrinsic part of the gametogenic program, and therefore a consequence not a cause of switching on the gametogenic program and CT genes in cancer.

In addition to questions about mechanisms for reactivating CT antigen expression in cancer, another important issue is whether expression of these genes in the cancer cell contributes to its malignant behavior? In the absence of specific information, discussion of this matter must be speculative, but the finding that gametes, trophoblasts and cancers share a battery of antigens restricted to these cell types is an invitation to extend the search for other shared characteristics. It was a similarity in the biological features of trophoblasts and cancer cells that prompted the Scottish embryologist John Beard at the turn of the last century to propose his trophoblastic theory of cancer (46, 47). In his view, cancers arise from germ cells that stray or are arrested in their trek to the gonads. Under the influence of carcinogenic stimuli, such cells undergo a conversion to malignant trophoblastic cells. These malignant trophoblastic cells take on features of the resident cell types in different organs, but the resulting cancers, no matter their site of origin or how distinct they appear morphologically, are of trophoblastic origin. Beard ascribed the invasive, destructive and metastatic features of cancer to functions normally displayed by trophoblastic cells, e.g., invasion of blood vessels, growth into the uterine wall, and spread beyond the uterus. From a contemporary perspective, Beard's idea that cancers are derived from arrested germ cells seems incompatible with our growing knowledge of serological and molecular markers that distinguish different pathways of normal differentiation and their preservation in cancer. Beard's insight that trophoblasts and cancer cells share common features is better explained by the induction of a gametogenic program in resident cancer cells, rather than the derivation of cancer from an aberrant germ cell. The end result, however, would be the same - selected features of cells undergoing gametogenesis and trophoblast development being imposed on transformed somatic cells.

In addition to CT antigens, what other features shared by germ cells and cancer might be expected? With the finding that SCP-1, a critical element in the meiotic program, is expressed in non-germ cell cancers, other proteins uniquely associated with meiosis should be sought. It goes without saying that the induction of a meiotic program in a somatic cell, normal or malignant, would likely lead to chromosomal anarchy, a prime feature of advanced cancers. Defining OY-TES-1, the proacrosin binding protein precursor that is part of the unique program leading to the formation of spermatozoa, as a CT antigen, suggests that other mature sperm-specific gene products might be expressed in cancer. In addition, expression of CT antigens by trophoblasts sheds new

light on an old issue - the much studied sporadic production of human chorionic gonadotropin (HCG) and other trophoblastic hormones by human cancers, e.g., (48, 49, 50). The production of HCG by cancer cells has been generally viewed as yet another indication of the genetic instability of cancer cells, resulting in the random and aberrant activation of silent genes during carcinogenesis and tumor progression. However, it can also be viewed as a consequence of the induction of a gametogenic/trophoblastic program in cancer, one that would also result in the semi-coordinate expression of CT antigens. Activation of this program would also confer other properties of germ cells, gametes, and trophoblasts on cancer cells, but these are more difficult to relate in any precise fashion. Nonetheless, immortalization, invasion, lack of adhesion, migratory behavior, induction of blood vessels, demethylation, and downregulation of MHC, are some features shared by cancer and by cells undergoing germ cell/gamete/trophoblast differentiation pathways. The metastatic properties of cancer may also have its counterpart in the migratory behavior of germ cells, and in the propensity of normal trophoblast cells to migrate to other organs, such as the lung, during normal pregnancy, but then to undergo involution at term.

In pursing the idea of a program change in cancer leading to the expression of gametogenic features, a hypothesis I call "Gametogenic Program Induction in Cancer" (GPIC), it might be well to distinguish at least four different pathways involved in germ cell development: A) germ cell to germ cell, B) germ cell to oogonia to oocytes, C) germ cell to spermatogonia to sperm, and D) germ cell to trophoblast. The meiotic program would be common to B and C, proteins like OY-TES-1 would be restricted to C, and HCG would be a characteristic of D. The reason for distinguishing these pathways and ultimately stages in each pathway is that the variety of patterns or sets of CT antigens observed in different cancers may be a reflection of the germ cell program, e.g., pathway and stage that has been induced in these cancers.

With this background and framework of thinking about the relation of gametogenesis and cancer development, there are a number of topics that need to be pursued:

1. The search for new CT antigens should be intensified. At present this can best be done by SEREX, particularly with libraries from testis, normal or malignant trophoblasts, or tumors or tumor cell lines (growing with or without demethylating agents) that express a range of CT antigens, or by extending the use of representational difference analysis. Bioinformatics and microarray technology offer exciting possibilities for mining cDNA databanks for transcripts that show cancer/gamete/trophoblast specificity.
2. Establishing the expression pattern of known CT antigens in normal gametogenesis and trophoblast development is critical for identifying markers that distinguish different pathways and stages in the normal gametogenic program. This information provides a basis for interpreting the complex patterns of CT expression in cancers in relation to gametogenic pathways/stages, and for suggesting new ways to classify cancer on the basis of CT phenotypes.
3. Although the frequency of expression of individual CT antigens in different tumor types has generally been defined, far more information is needed about the composite CT phenotype of individual tumors, and how frequently these composite CT patterns are seen in tumors of different origin. With this information, we can begin to establish whether there is any correlation with other biological features of the tumor, e.g., growth rate, local vs. invasive, primary vs. metastatic, different metastatic deposits in the same patient, etc.
4. At which stage in the life history of cancer are CT (gametogenic) features induced? This can be approached in model systems in the mouse, in vitro systems with human cells, or with naturally occurring tumors in man that show incremental stages in tumor progression. As discussed above, there is evidence that CT expression is a sign of greater malignancy, but this needs to be more firmly established in a broad range of tumor types.
5. The heterogeneous expression of CT antigens in a large proportion of human cancers needs to be understood. Does this reflect a quantitative difference in levels of mRNA/protein in CT^+ and CT^- cells, or is there a qualitative distinction between CT^+ and CT^- cells in CT mRNA/protein expression? Laser dissection microscopy may be one way to analyze this question and cloning of tumor cells from a tumor with heterogeneous CT expression could be another approach. There is a growing impression that established human cancer cell lines show a higher frequency of CT antigen expression than what would be expected from CT typing of the corresponding tumor type, particularly tumors with a low frequency of CT expression. This could be a secondary consequence of *in vitro* culture, or it could be that CT^+ cells (even if they represent only a minority population of the tumor) have a growth advantage for propagating *in vitro*, and possibly also *in vivo*.
6. Although CT antigens provide a strong link between the gametogenic program and cancer, it will be critical to determine whether other distinguishing features of gamete development are expressed by

cancer and whether their expression can be correlated with CT antigen expression. The many reports over the last three decades of HCG production by certain human cancers provide a specific starting point to explore this issue and ask whether the production of HCG can be correlated with CT antigen expression, particularly a unique pattern of CT expression, one possibly reflecting the trophoblast program.

7. Defining the role of CT antigens in gametogenesis and trophoblast development and their functional significance in cancer represents major challenges. Transgenic and knock-out approaches using mouse CT counterparts, and transfection analysis with CT coding genes in normal and malignant human cells are obvious ways to begin addressing these issues. However, the multiplicity of CT antigens, their likely overlapping activities, and the problem of devising relevant assay systems, suggest that deciphering the functional meaning of CT antigens in cancer will not be an easy task.

Much of contemporary cancer research is dominated by the search for abnormalities in growth regulatory genes and for understanding how the individual and composite activities of these genes result in the phenomenon we call cancer. Genetic changes in cancer also have the potential for re-awakening silent genetic programs in cancer cells, conferring functions and behavior that are normally expressed at other places and at other times in the organism, and that are advantageous to the success of cancer. The basis for the idea that a gametogenic program is induced in cancer comes from the discovery of a battery of CT antigens that normally show highly restricted expression in gametes and trophoblasts. The idea that gametic / trophoblastic traits are re-expressed in cancer is certainly not a new one, nor one that has escaped the interest of many investigators. However, with the array of powerful new techniques at hand, it is now possible to establish whether cancer has acquired some of its malignant properties by commandeering the gene program that initiates life.

Abbreviations

CT, cancer/testis; HCG, human chorionic gonadotropin; SEREX, serological analysis of cDNA expression libraries

References

1. Boyse EA, Miyazawa M, Aoki T, Old LJ. Ly-A and Ly-B: two systems of lymphocyte isoantigens in the mouse. *Proc Royal Soc Brit* 1968; **170**: 175-193. (PMID: 4385242)

2. Boyse EA, Old LJ. Some aspects of normal and abnormal cell surface genetics. *Ann Rev Genet* 1969; **3**: 269-290.

3. DeLeo AB, Jay G, Appella E, Dubois GC, Law LW, Old LJ. Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. *Proc Natl Acad Sci USA* 1979; **76**: 2420-2424. (PMID: 221923)

4. Lilly F, Boyse EA, Old LJ. Genetic basis of susceptibility to viral leukemogenesis. *Lancet* 1965; ii:1207-1209.

5. Carswell EA, Old LJ, Kassel RL, Green S, Fiore NC, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA* 1975; **72**: 3666-3670. (PMID: 1103152)

6. Traversari C, van der Bruggen P, Van den Eynde B, Hainaut P, Lemoine C, Ohta N, Old LJ, Boon T. Transfection and expression of a gene coding for a human melanoma antigen recognized by autologous cytolytic T lymphocytes. *Immunogenetics* 1992; **35**: 145-152. (PMID: 1537606)

7. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, and Boon, T, A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 1991; **254**:1643-1647. (PMID: 1840703)

8. Old LJ. Cancer immunology: The search for specificity. *Cancer Res* 1981; **41**: 361-375. (PMID: 7004632)

9. Knuth A, Danowski B, Oettgen HF, Old LJ. T cell-mediated cytotoxicity against malignant melanoma: Analysis with IL-2-dependent T cell cultures. *Proc Natl Acad Sci USA* 1984; **81**: 3511-3515. (PMID: 6610177)

10. Sahin, U, Türeci Ö, Schmitt, Cochlovius B, Johannes T, Schmits R, Stenner F, Luo G, Schobert I, Pfreundschuh M. Human neoplasms elicit multiple specific immune responses in the autologous host. *Proc Natl Acad Sci USA* 1995; **92**: 11810-11813. (PMID: 8524854)

11. Old LJ, Chen YT. New paths in human cancer serology. *J Exp Med* 1998; **187**: 1163-1167. (PMID: 9547328)

12. De Plaen E, Arden K, Traversari C, Gaforio JJ, Szikora JP, De Smet C, Brasseur F, van der Bruggen P, Lethé B, Lurquin C, Brasseur R, Chomez

P, De Backer O, Cavenee W, and Boon T. Structure, chromosomal localization and expression of twelve genes of the MAGE family. *Immunogenetics* 1994; **40**: 360-369. (PMID: 7927540)

13. Muscatelli F, Walker AP, De Plaen E, Stafford AN, Monaco AP. Isolation and characterization of a new MAGE gene family in the Xp21.3 region. *Proc Natl Acad Sci USA* 1995; **92**: 4987-4991. (PMID: 7761436)

14. Boël P, Wildmann C, Sensi ML, Brasseur R, Renauld JC, Coulie P, Boon T, van der Bruggen P. BAGE, a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. *Immunity* 1995; **2**: 167-175. (PMID: 7895173)

15. Van den Eynde B, Peeters O, De Backer O, Gaugler B, Lucas S, Boon T. A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. *J Exp Med* 1995;**182**: 689-698. (PMID: 7544395)

16. De Backer O, Arden KC , Boretti M, Vantomee V, De Smet C, Czekay S, Viars CS, De Plaen E, Brasseur F, Chomez P, Van den Eynde B, Boon T, van der Bruggen P. Characterization of the GAGE genes that are expressed in various human cancer and in normal testis. *Cancer Res* 1999; **59**: 3157-65. (PMID: 10397259)

17. Güre AO, Türeci Ö, Sahin U, Tsang S, Scanlan MJ, Jager E, Knuth A, Pfreundschuh M, Old LJ, Chen YT. SSX, a multigene family with several members transcribed in normal testis and human cancer. *Int. J. Cancer* 1997; **72**: 965-971. (PMID: 9378559)

18. Chen YT, Scanlan MJ, Sahin U, Türeci Ö, Güre AO, Tsang S, Williamson B, Stockert E, Pfreundschuh M, Old LJ. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc. Natl. Acad. Sci USA* 1997; **94**: 1914-1918. (PMID: 9050879)

19. Lethe B, Lucas S, Michaux L, De Smet C, Godelaine D, Serrano A, De Plaen E, Boon T. LAGE-1: a new gene with tumor specificity. *Int J Cancer* 1998;**76**: 903-908. (PMID: 9626360)

20. Türeci Ö, Dahin U, Zwick C, Koslowski M, Switz G, Pfreundschuh M. Identification of a meiosis-specific protein as a new member of the class of cancer/testis antigens. *Proc Natl Acad Sci USA* 1998; **95**: 5211-5216. (PMID: 9560255)

21. Chen YT, Güre AO, Tsang S, Stockert E, Jäger E, Knuth A, Old LJ. Identification of multiple cancer/testis antigens by allogeneic antibody screening of a melanoma cell line library. *Proc Natl Acad Sci USA* 1998; **95**: 6919-6923. (PMID: 9618514)

22. Lucas S, De Smet C, Arden KC, Viars CS, Lethe B, Lurquin C, Boon T. Identification of a new MAGE gene with tumor-specific expression by representational difference analysis. *Cancer Res* 1998; **58**: 743-752. (PMID: 9485030)

23. Sahin U, Koslowski M., Türeci Ö, Eberle T, Zwick C, Romeike B, Moringlane JR, Schwechheimer K, Feiden W., Pfreundschuh M. Expression of cancer/testis genes in human brain tumors. *Clin Cancer Res* 2000;**10**: 3916-3922. (PMID: 11051238)

24. Scanlan MJ, Altorki NK, Güre AO, Williamson B, Jungbluth A, Chen YT, Old LJ. Expression of cancer-testis antigens in lung cancer: definition of bromodomain testis-specific gene (BRDT) as a new CT gene CT9. *Cancer Lett*. 2000; **150**: 155-164. (PMID: 10704737)

25. Güre AO, Stockert E, Arden KC, Boyer AD, Viars CS, Scanlan MJ, Old LJ, Chen YT. CT10: a new cancer-testis (CT) antigen homologous to CT7 and the MAGE family, identified by representational difference analysis. *Int J Cancer* 2000; **85**: 726-732. (PMID: 10699956)

26. Lucas S, De Plaen E, Boon T. MAGE-B5, MAGE-B6, MAGE-C2 and MAGE-C3: four new member of the MAGE family with tumor-specific expression. *Int J Cancer* 2000; **87**: 55-60. (PMID: 10861452)

27. Zendman AJ, Cornelissen IM, Weidle UH, Ruiter DJ, van Muijen GN. CTp11, a novel member of the family of human cancer/testis antigens. *Cancer Res* 1999, **59**: 6223-6239. (PMID: 10626816)

28. Martelange V, De Smet C, De Palen E, Lurquin C, Boon, T. Identification on a human sarcoma of two new genes with tumor-specific expression. *Cancer Res* 2000; **60**: 3848-3855. (PMID: 10919659)

29. Eichmüller S, Usener D, Dummer R, Stein A, Thiel D, Schadendorf D. Serological detection of cutaneous T cell lymphoma-associated antigens. *Proc Natl Acad Sci USA* 2001; **98**: 629-634. (PMID: 11149944)

30. Ono T, Kurashige T, Harada N, Noguchi Y, Saika T, Niikawa N, Aoe M, Nakamura S, Higashi T, Hiraki A, Wada H, Kumon H, Old L, Nakayama E. Identification of proacrosin binding protein sp32 precursor as a human cancer/testis antigen. *Proc Natl Acad Sci.* 2001; **98**: 3282-3287. (PMID: 11248070)

31. Jungbluth A, Busam K, Kolb D, Iversen K, Coplan K, Chen YT, Spagnoli GC, Old LJ. Expression of MAGE-antigens in normal tissues and cancer. *Int J Cancer*. 2000; **85**: 460-5. (PMID: 10699915)

32. Jungbluth A, Busam K, Iversen, K, Kolb D, Coplan K, Chen YT, Stockert E, Zhang P, Old, LJ. Cancer-Testis (CT) antigens MAGE-1, MAGE-3, NY-ESO-1, and CT7 are expressed in female germ cells. *Mod Path*. In press 2001.

33. Jungbluth A, Iversen K, Kolb D, Coplan K, Chen YT, Stockert E, Old LJ, Vogel M. Expression of CT (Cancer/Testis) antigens MAGE, NY-ESO-1, and CT7 in placenta. *German Soc for Path*. Submitted 2001.

34. Jungbluth A, Stockert E, Chen YT, Kolb D, Iversen K, Coplan K, Williamson B, Altorki N, Busam KJ, Old LJ. Monoclonal antibody MA454 reveals a heterogeneous expression pattern of MAGE-1 antigen in formalin-fixed paraffin embedded lung tumours. *British Journ Cancer* 2000; **83**: 493-497. (PMID: 10945497)

35. Meuwissen RJL, Offenberg, HH, Dietrich AJ, Riesewijk A, van Iersel M, Heting C. A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. *EMBO J* 1992;**11**: 5091-5100. (PMID: 1464329)

36. Baba T, Niida Y, Michikawa Y, Kashiwabara S, Kodaira K, Takenaka M, Kohno N, Gerton GL, Arai Y. An acrosomal protein, sp32, in mammalian sperm is a binding protein specific for two proacrosins and an acrosin intermediate. *J Biochem* 1994; **269**: 10133-10140. (PMID: 8144514)

37. Brasseur F, Rimoldi D, Liénard D, Lethe B, Carrel S, Arienti F, Suter L, Vanwijck R, Bourlond A, Humblet Y, Vacca A, Conese M, Lahaye T, Degiovanni G, Deraemaecker R, Beauduin M, Sastre X, Salamon E, Dréno B, Jäger E, Knuth A, Chevreau C, Suciu S, Lachapelle J-M, Pouillart P, Parmiani G, Lejeune F, Cerottini J-C, Boon T, Marchand M. Expression of MAGE gene in primary and metastatic cutaneous melanoma. *Int J Cancer* 1995; **63**: 375-380. (PMID: 7591235)

38. Patard JJ, Brasseur F, Gil-Diez S, Radvanyi F, Marchand M, Francois P, Abi-Aad A, VanCangh P, Abbou CC, Chopin D, Boon T. Expression of MAGE genes in transitional-cell carcinomas of the urinary bladder. *Int J Cancer* 1995; **64**: 60-64. (PMID: 7665250)

39. Kurashige T, Noguchi Y, Saika T, Ono T, Nagata Y, Jungluth A, Ritter G, Chen YT, Stockert E, Tomoyasu T, Kumon H, Old LJ, Nakayama E. NY-ESO-1 expression and immunogenicity associated with transitional cell carcinoma: correlation with tumor grade. Submitted 2001.

40. Stockert E., Jäger E, Chen YT, Scanlan M, Gout I, Karbach J, Arand M, Knuth A, Old, LJ. A survey of the humoral response of cancer patients to a panel of human tumor antigens. *J Exp Med* 1998;**187**: 1349-1354. (PMID: 9547346)

41. Jäger E., Nagata Y, Gnjatic S, Wada H, Stockert E, Karbach J, Dunbar PR, Lee SY, Jungbluth A, Jäger D, Arand M, Ritter G, Cerundolo V, Dupont B, Chen YT, Old LJ, Knuth A. Monitoring CD8 T cell responses to NY-ESO-1: Correlation of humoral and cellular immune responses. *Proc Natl Acad Sci USA* 2000; **97**: 4760-4765. (PMID: 10781081)

42. De Smet C, De Backer O, Faraoni I, Lurquin C, Brasseur F, Boon T. The activation of human gene MAGE-1 in tumor cells is correlated with geneome-wide demethylation. *Proc. Natl. Acad. Sci USA* 1996; **93**: 7149-7153 (PMID: 8692960)

43. Jungbluth A, Chen YT, Stockert E, Busam KJ, Kolb D, Iversen K, Coplan K, Williamson B, Altorki N, Old LJ. Immunohistochemical analysis of NY-ESO-1 antigen expression in normal and malignant tumors. *Int J Cancer.* In press 2001.

44. Jungbluth A, Antonescu C, Busam K, Iversen K, Kolb D, Coplan K, Chen YT, Stockert E, Ladanyi M, Old, LJ. Monophasic and biphasic synovial sarcomas abundantly express cancer/testis antigen NY-ESO-1, but not MAGE-A1 or CT7. *Int Jour Cancer*. Submitted 2001.

45. Antonescu C, Busam K, Iversen K, Kolb D, Coplan K, Spagnoli G, Ladanyi M, Old LJ, Jungbluth, A. MAGE antigen expression in monophasic and biphasic synovial sarcoma. *Mod Path.* Submitted 2001.

46. Beard J. The cancer problem. *Lancet* 1905;**1**:281-203.

47. Gurchot C. The trophoblast theory of cancer. *Oncology* 1975; **31**: 310-333. (PMID: 1107920)

48. Iles RK, Chard T. Human Chorionic Gonadotropin Expression by Bladder Cancers: Biology and Clinical Potential. *J Urol* 1991; **145**: 453-458. (PMID: 1705292)

49. Acevedo HF, Tong JY, Hartsock RJ. Human Chorionic Gonadotropin-Beta Subunit Gene Expression in Cultured Human Fetal and Cancer Cells of Different Types and Origins. *Cancer* 1995; **76**: 1467-1475. (PMID: 8620425)

50. Dirnhofer S, Koessler P, Ensigner C, Feichtinger H, Madersbacher S, Berger P. Production of Trophoblastic Hormones by Transitional Cell Carcinoma of the Bladder: Association to Tumor Stage and Grade. *Hum Path* 1998; **29**: 377-382. (PMID: 9563788)

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